Chapter 1
Introduction

1. Diabetes

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Clinically, DM is divided into two major classes: diabetes mellitus Type 1 and Type 2. Until 1998, Type 1 and Type 2 were named insulin-dependent diabetes mellitus (IDDM) and noninsulin dependent diabetes mellitus (NIDDM) respectively. Diabetes mellitus constitutes a growing global public health problem, from 30 million people affected 10 years ago to about 135 million at present (1), and an estimated 300 million by 2025 [World Health Organization (WHO) 1997]. This global epidemic is not only confined to the industrialized world but is also increasing in less developed countries where urbanization and industrialization are proceeding rapidly (1, 2). A population of developing countries, minority groups and disadvantaged communities in industrialized countries is now facing the greatest risk (3, 4). This arising condition has been called a ‘new world syndrome’ (2), a symptom of globalization and its social, cultural, economic and political significance (4). The pandemic basically involves Type 2 diabetes which comprises about 85% of all DM cases (2). DM not only has adverse impact on health, but also on the economic cost. Their complications are enormous, both in health care and loss of productivity to society (4). It has been estimated that in the future, DM will constitute a heavy burden both for individuals affected and for the societies in which they live (5). The term epidemic is generally linked to the communicable and infectious diseases rather than non communicable diseases such as diabetes mellitus. At present it is becoming a big question to why is DM increasing to epidemic proportions and what can be done to stop this increase. Efforts are being taken on employing new research findings and aggressive strategies to fight this fatal disease (6).

The World Health Organization recognizes three main forms of diabetes mellitus:

1) Type1 diabetes
2) Type2 diabetes
1.1. Type 1 diabetes
In type 1 diabetes, the insulin-producing beta cells are destroyed by the body's own immune system. It develops most often in children and young but can appear at any age. Symptoms of type 1 diabetes usually develop over a short period, although beta cell destruction can begin years earlier. Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person with type 1 diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis.

1.2. Type 2 diabetes
Type 2 diabetes occurs due to defects in the islet beta cells, and to an impairment of insulin's ability to stimulate the uptake of glucose in muscles and other tissues. The cause of this insulin resistance has not yet been fully established, but may involve defects in the action of insulin after it has bound to the insulin receptor GLUT on the surface of cells. There is a genetic influence, as type 2 diabetes tends to run in families even more strongly than type 1 diabetes, and several genes are likely to be involved. But increasing age, obesity and a sedentary lifestyle also increases the risk of type 2 diabetes. It is most often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities (7). About 80 to 90% of people suffer from this type of diabetes worldwide. Type 2 diabetes is increasingly being diagnosed in children and adolescents. The symptoms of type 2 diabetes develop gradually. Their onset is not as sudden as in type 1 diabetes. Symptoms may include fatigue, frequent urination, increased thirst and hunger, weight loss, blurred vision, and slow healing of wounds or sores. Some people may not develop any symptoms.

1.3. Gestational diabetes
Some women develop gestational diabetes in late pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had
gestational diabetes have a 20 to 50% chance of developing type 2 diabetes within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent the development of type 2 diabetes. As with type 2 diabetes, gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Gestational diabetes is caused by the hormones of pregnancy or a shortage of insulin (8). Women with gestational diabetes may not experience any symptoms.

2. Complications associated with diabetes.

The occurrence of systemic hyperglycemia in diabetes can ultimately cause long-term damage to multiple organs and lead to severe complications. The microvasculature is a key target of hyperglycemic damage. It damages the small blood vessels which lead to systemic complications. Among these complications is the development of cardiovascular disease. Diabetic cardiovascular injury includes both cardiac and peripheral blood vessels. However, research has focused largely on peripheral vascular injury. Retinopathy (disease of the retina that can lead to vision loss), neuropathy (nerve damage), and nephropathy (kidney disease) are all caused, in part, by changes in blood flow or abnormal vessel growth (angiogenesis).

2.1. Neuropathy

A widely accepted definition of diabetic peripheral neuropathy is "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes (9). Diabetic neuropathy is classified into several syndromes, each with a distinct pattern of involvement of peripheral nerves. Patients often have multiple or overlapping syndromes. Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time develop nerve damage throughout the body. Some people with nerve damage have symptoms such as pain, tingling, or numbness, loss of feeling in the hands, arms, feet, and legs. Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs. About 60 to 70% of people with diabetes have some form of neuropathy. People with diabetes can develop nerve problems at any time, but risk rises with age and longer duration of diabetes.
2.2. Retinopathy
A well-recognised and common complication of diabetes is damage to the blood vessels in the retina, the nerve fibre layer at the back of the eye. This is known as retinopathy and is the largest single cause of blindness amongst adults (10). Diabetes can cause the small blood vessels in the retina to become blocked and damaged. This can result in leaking of small amount of blood and the retina is starved of oxygen. This is known as background non-proliferative diabetic retinopathy. If enough small blood vessels become blocked, new blood vessels start to grow within the eye in an attempt to provide oxygen to the damaged retina. This is known as proliferative retinopathy and initially it has no effect on vision. However, the new blood vessels created within the eye are generally weak and grow into the vitreous, the jelly-like substance inside the eyeball. The weak vessels can bleed (vitreous haemorrhage) and/or pull the retina off the back of the eye (retinal detachment) resulting in loss of vision. Loss of vision may occur if the centre of the retina, known as the macula, is affected by diabetic retinopathy. Diabetic retinopathy is the third most common cause of blindness and the leading cause of new blindness in individuals 20–74 years of age (11). In the 1990s, randomized trials confirmed that control of hyperglycemia and hypertension could prevent retinopathy (12, 13).

2.3. Nephropathy
Diabetes has become the most common single cause of end-stage renal disease (ESRD) in the human population. About 20–30% of patients with type 1 or type 2 diabetes develop evidence of nephropathy, but in type 2 diabetes, a considerably smaller fraction of these progress to ESRD. ESRD from diabetes persists as a huge cause of patient morbidity. The pathology of diabetic nephropathy manifests histologically as diabetic glomerulosclerosis, and is characterized by glomerular basement membrane thickening and mesangial expansion with increased extra cellular matrix deposition. In Type I diabetics, there is a direct relationship between the extent of mesangial expansion and clinical severity of disease (14). In this regard, Mauer’s seminal paper demonstrated a direct correlation between the degree of mesangial expansion, and magnitude of proteinuria, severity of
hypertension, and degree of renal impairment (14). Mesangial expansion in diabetic glomerulosclerosis may be considered the result of an imbalance between mesangial matrix protein production and degradation, favoring matrix protein accumulation. Thus, the mediators of mesangial expansion constitute reasonable therapeutic targets when crafting a treatment strategy for diabetic nephropathy.

2.4. Cardiomyopathy

The term “specific cardiomyopathy” is now used to describe heart muscle diseases that are associated with specific cardiac or system disorders. Diabetic cardiomyopathy, as one type of specific cardiomyopathy, is a diabetes-related myopathy and is characterized mainly by impaired diastolic function (15, 16, 17, 18). Among these complications is the development of cardiovascular disease. It has been estimated that 65–70% of diabetics die of heart disease, making it the major mortality in both Type 1 and Type 2 diabetes. Rubler et al. first recognized the existence of diabetic cardiomyopathy in diabetic patients with congestive heart failure (19). After a myocardial infarction, diabetic patients are twice as likely to die and three times more likely to progress to congestive heart failure than are nondiabetic patients (20). The Framingham study (21) of 292 diabetic and 4900 nondiabetic subjects and a recent study (22) of 1810 diabetic patients and 944 age-matched controls further demonstrated significantly increased incidence of heart failure in diabetics, irrespective of coronary artery diseases and hypertension. Moreover, diabetes accelerates heart failure to a greater extent in women than in men. Taken together, these studies show that diabetes mellitus affects cardiac structure and function independent of blood pressure or coronary artery disease, although hypertension can accelerate myocardial damage in diabetic patients (15, 16, 23, 24). The development of diabetic cardiomyopathy is associated with defects in cellular organelles such as myofibrils, mitochondria, sarcoplasmic reticulum, and sarcolemma. In addition, the calcium-handling properties of the diabetic heart are altered as a consequence of changes in myocardial metabolism, and these then determine the derangement of processes involved in cardiac contraction and relaxation. This derangement will eventually lead to heart dysfunction and heart failure (25).
Although recent studies have extended our understanding of the pathogenesis of the vascular complications of diabetes, the mechanism by which hyperglycemia causes tissue damage, especially cardiomyopathy, is poorly understood (15, 16, 26).

3. Mechanism of diabetic cardiomyopathy

Several studies have shown that hyperglycemia as an independent risk factor directly causes cardiac damage, lending to diabetic cardiomyopathy (19, 22, 25, 27). However, mechanisms for the pathogenesis remain unclear (15, 19, 22, 25, 27). Diabetic hearts in streptozotocin (STZ) induced diabetic animal models, display a reduction in cardiac mass over time, myocardial hypertrophy and interstitial and perivascular fibrosis at late phase (15, 28). These late-phase changes are believed to result from early responses of myocardium to suddenly increased glucose levels (25, 28, 29, 30). Early responses of myocardial cells to hyperglycemia include metabolic abnormalities, subcellular defects, abnormal expression of genes (29, 30), and consequently, cardiac cell death (30, 31, 32). Cell death, as a comprehensive consequence of myocardial abnormalities, is an important cause of cardiomyopathy (31, 32, 33). In particular, cell death can cause a loss of contractile tissue, compensatory hypertrophy of myocardial cells, and reparative fibrosis (31). Diabetes-induced cell death has been observed in multiple organs in vivo (34, 35, 36) and in endothelial cells in vitro (37, 38, 39). Recent studies showed that the incidence of apoptosis increases in the heart of patients with diabetes (40) and STZ-induced diabetic animals (41, 42). However, whether the increased myocardial apoptotic cell death is related directly to hyperglycemia is unclear.

3.1. Diabetic cardiomyopathy in type 1 diabetes

Type 1 diabetes mellitus leads to a cardiomyopathy in both human and animal models. The existence of a diabetic cardiomyopathy in humans is based on the presentation of ventricular dysfunction in patients without evidence of any other known cardiovascular disease (19, 43, 44). Numerous studies carried out since 1972 have supported the view that diabetic cardiomyopathy is a pervasive problem in type 1 diabetes and is first manifested by diastolic dysfunction (45).
This cardiomyopathy is well described in animal models with long-term type 1 diabetes and results in abnormal cardiomyocyte excitation-contraction (E-C) coupling (26, 46). The cellular mechanisms that contribute to myocyte dysfunction involve depressed expression and function of sarcoplasmic reticulum calcium ATPase (SERCA) and Na+/Ca2+ exchanger (47). Regulation of E-C coupling is also impaired in diabetic hearts, such that β-adrenergic receptor signaling is depressed, which may result from changes in β-adrenergic receptor density or redistribution of β-adrenergic receptor subtypes (48), or perhaps signaling downstream of the receptors (49). Elevated protein kinase C (PKC) activity and changes in the expression of specific PKC isoforms are also found in type I diabetic hearts (50, 51).

3.2. Diabetic cardiomyopathy in type 2 diabetes
Diabetic cardiomyopathy has also been determined clinically in the type 2 diabetic patients. Investigators in the Strong Heart Study (SHS) performed detailed echocardiographic (ECG) analysis of a population of American Indians with a high rate of type 2 diabetes and reported that diabetes was associated with increased left ventricular (LV) mass, LV wall thickness, reduced systolic and particularly diastolic function, independent of hypertension (22, 52). Similar to diabetes, investigators from the Framingham Heart Study reported increased LV mass and wall thickness in individuals with glucose intolerance and insulin resistance (53). There was also an association between left atrial (LA) size and insulin resistance. Several investigators have experimentally shown that diabetes mellitus is associated with a specific cardiomyopathy (54, 55). Impaired insulin action (ie, insulin resistance) is characterized by a compensatory hyperinsulinemia and hyperlipidemia, which are major metabolic dysfunctions, associated with the early stages of type 2 diabetes. Elevated plasma insulin and lipid levels can lead to numerous metabolic and pathophysiological derangements in various tissues, including the heart. Abnormal ventricular systolic and diastolic functions are reported in type 2 patients presenting without macrovascular disease or hypertension, providing indirect evidence that there is a diabetic cardiomyopathy in humans (56).
4. Stages of diabetic cardiac effects

4.1. Early responses of myocardial cells to hyperglycemia

The presence of several factors in diabetes leads to high occurrence of cardiovascular disease (CVD) such as hyperglycemia, insulin resistance, and classical and non-classical risk factors (dyslipidemia, ketone bodies, proinflammatory conditions and oxidative stress) lead to high occurrence of cardiac damage and apoptosis.

As shown in Fig. 1, typical changes in membrane and contractile function usually occur days after diabetic development, whereas morphological changes and heart dysfunction take months to years. The chronic alterations at the late stages of diabetes are believed to be result of acute cardiac responses arises due to sudden increase in glucose levels at the early stage (29, 33).

4.2. Middle stage of myocardial cells to hyperglycemia

Cellular changes such as defects in calcium transport and fatty acid metabolism may lead to increases in myocyte apoptosis and necrosis, angiotensin II, TGF-β1, and resulting in myocyte injury, loss and myocardial fibrosis and initially causing abnormal mitral inflows that may advance to low ejection fraction. This stage of diabetic cardiomyopathy is mainly characterized by myocellular hypertrophy and myocardial fibrosis (Fig. 1). Patients at this stage may have minor changes in structure (such as LV dimension, wall thickness, or mass) and significant changes in diastolic and systolic function, which may be detected by conventional echocardiography. Myocardial vascular structural lesions at this stage are usually insignificant.

4.3. Late stage of myocardial cells to hyperglycemia

The further changes in metabolism and development of myocardial fibrosis result in myocardial microvascular changes. This stage of diabetic cardiomyopathy is characterized by myocardial microvascular structural and functional changes accompanying recurrent micro vascular spasm (Fig. 1). Changes in cardiac structure and function are obvious. Diabetic cardiomyopathy at this stage is frequently associated with hypertension and early development of ischemic heart disease in diabetes.
5. Metabolic abnormalities in the diabetic heart

5.1. Insulin

5.2. Increased lipid metabolism

5.3. Dyslipidemia

5.4. Ketone bodies

5. Metabolic abnormalities in the diabetic heart

Myocardial Energy Metabolism Cardiac function demands a high amount of energy supplied as adenosine triphosphate (ATP). Under normal physiological conditions, the adult heart uses a combination of (80%) glucose, (20%) free fatty acids (FFA), pyruvate, and ketone bodies as fuel sources (28, 57). In uncontrolled diabetes, the use of glucose decreases, and FFA oxidation can provide from 90% to 100% of the heart’s ATP requirements (28) (Fig. 2). Glucose metabolism can be roughly divided into three categories: (I) glucose transport and phosphorylation, (II) glycolysis (glucose metabolism from glucose-
6-phosphate to pyruvate and lactate), and (III) glucose oxidation. All aspects of glucose metabolism with the exception of glycolysis are inhibited in diabetes; the diabetic heart mostly relies on the oxidation of lipids for its energy support (28). Glucose uptake is mediated by glucose transporter protein (GLUT). GLUT4 is found primarily in insulin-sensitive tissue such as fat and skeletal muscle and is the predominant isoforms of cardiac muscle. Decreased glucose transport is directly related to the decrease in abundance of GLUT4 mRNA and its protein (28, 58, 59) (Fig. 2). In experimentally induced diabetic animal models, the levels of GLUT4 mRNA and protein were reduced by about more than 50%, and both basal and the insulin-stimulated glucose transport were significantly reduced in their cardiomyocytes (28, 57).

Fig. 2: Metabolic abnormalities in the diabetic heart: Impaired in insulin signaling leading to the decreased the glucose uptake and increased FA oxidation. Increased FA oxidation leads to the generation of ROS at the level of the electron transport chain. ROS, which also can be generated by extra-mitochondrial mechanisms such as NADPH oxidase, plays a critical role in several pathways involved in the pathogenesis of diabetic cardiomyopathy, including lipotoxicity, cell death, and tissue damage, as well as mitochondrial uncoupling and reduced cardiac efficiency. [Adapted from Circulation, 2007; 115:3213-23(60)]
5.1. Insulin

Insulin signaling is an important regulator of substrate metabolism in vertebrates in the heart (61). Insulin has direct effects on glucose transport (62), glycolysis (63), glucose oxidation (64), glycogen synthesis (65), and protein synthesis (66). Insulin may also increase cardiac contractility (67) and may have an antiapoptotic effect on cardiomyocytes (68). In vivo, many of the effects of insulin on cardiac metabolism and function are related to the systemic effects of insulin, such as increased peripheral and coronary vasodilatation (69, 70), increased sodium and water uptake by the kidneys (71), changes in the delivery of substrates to the heart (72), and reduce fatty acid oxidation rates (73). Diminished glucose oxidation rates in cardiomyocytes occur as early as 48 hr after the induction of diabetes by STZ (74), and impaired transcription of the major cardiac glucose transporter GLUT4 is seen within 4 days after the induction of diabetes (75). Reversal of these early changes by insulin administration suggests that impaired or absent insulin signaling may play a central role in the mechanism of these alterations. Furthermore, in hyperinsulinemic animal models of type 2 diabetes such as the Zucker diabetic (fa/fa) rat, impaired insulin signal transduction in cardiac muscle (76) is also associated with diminished glucose utilization and increased fatty acid utilization in the heart (77). In an attempt to dissect the complex pathophysiology of insulin action in the heart, they have used cre/loxP recombination to specifically inactivate insulin signaling in cardiac myocytes in vivo in mice, while preserving insulin signaling in other cells such as endothelial cells, vascular smooth muscle cells, and liver and skeletal muscle cells (62, 78).

5.2. Increased lipid metabolism

More than 64% of type 1 and 34% of type 2 diabetic patients have low cardiac glucose uptake (79, 80). Glucose is the primary energy source for the heart during metabolic stress such as ischemia or hypoxia (81). Studies have shown that enhanced glucose uptake, by provision of an energy source for ATP synthesis, has a beneficial effect on contractile function and coronary flow during ischemia (81). Conversely, failure to glucose uptake and to maintain ATP levels during hypoxia is associated with increased cardiomyocyte death (82, 83). Diminished glucose utilization and contractile dysfunction have been reported in the heart of db/db mice, Zucker diabetic (fa/fa) rat, a model of type 2 diabetes (76, 84). These
abnormalities were corrected by selective overexpression of GLUT4 in the heart, the major glucose transporter expressed in this tissue (84).

5.3. Dyslipidemia
Growing evidence suggests that dyslipidemia contributes significantly to the excess risk of cardio-vascular disease (85). Lipid-lowering therapy can slow the progression of atherosclerosis and decrease the risk for cardiovascular events in patients with diabetes (86). Common characteristic features of diabetic dyslipidemia are the elevation of plasma triglycerides and triglyceride-rich VLDL cholesterol, reduced HDL cholesterol, and an increased number of small dense LDL cholesterol particles (85).

A full lipid profile shows the actual levels of each type of fat in the blood, cholesterol, LDL, HDL, VLDL, triglycerides, and others. Cholesterol and other fats are transported through the bloodstream in the form of round particles called lipoproteins. Unhealthy cholesterol, particularly low-density lipoprotein (LDL), forms a fatty substance called plaque, if it can contribute to the formation of plaque build up in the arteries, known as atherosclerosis. Smaller plaques remain soft, but older, larger plaques tend to develop fibrous caps with calcium deposits. Eventually these calcified and inelastic arteries become narrower (a condition known as stenosis). If a clot (thrombus) blocks an artery as this process continues, blood flow slows and prevents sufficient oxygen-rich blood from reaching the heart. This condition leads to angina (chest pain) and, in severe, cases to heart attack (if the occlusion occurs in the heart), or a stroke (if it occurs in the brain).

High cholesterol is at higher risk for developing heart disease as adults. Elevated cholesterol levels early in life may play a role in the development of adult atherosclerosis. High density lipoproteins (HDL) and is a type of fat in the blood that helps to remove cholesterol from the blood, preventing the fatty build up and formation of plaque.

Triglycerides are another class of fat found in the bloodstream. The bulk of the body fat tissue is in the form of triglycerides. The link between triglycerides and heart disease is under clinical investigation. However, many children with high triglyceride levels also have other risk factors such as high LDL levels or low HDL levels. Elevated triglyceride levels may be caused by medical conditions such as diabetes, hypothyroidism, kidney disease, or liver disease. Dietary causes
of elevated triglyceride levels may include obesity and high intakes of fat, alcohol, and concentrated sweets. Deposited LDL undergoes modification as free radicals oxidize LDL to form foam cells that form a thick, hard plaque. Low-density lipoprotein (LDL) transports about 75% of the blood's cholesterol to the body's cells, it is normally harmless. However, if it is exposed to a process called oxidation, it can penetrate and interact dangerously with the walls of the artery, producing a harmful inflammatory response. In response to oxidized LDL, the body releases various immune factors aimed at protecting the damaged walls. Unfortunately, in excessive quantities they cause inflammation and promote further injury to the areas they target. Immune factors that increase the risk for blood clots are also mobilized. The harmful imbalance of high triglycerides with low HDL levels is also associated with obesity (particularly around the abdomen), insulin resistance, and diabetes.

5.4. Ketone bodies
Elevated blood levels of the ketone bodies (hyperketonemia) acetoacetate (AA), β-hydroxybutyrate (BHB) and acetone (ACE) are encountered in Type-I diabetic patients and increased incidence of vascular disease, morbidity, and mortality (87). In diabetes, the level of ketone bodies begins to rise in the blood (ketonemia) because body fuel is mainly derived from fat (88). The blood concentration of ketone bodies may reach 10 mM in diabetic patients with severe ketosis compared with concentrations of less than 0.5 mM in normal human (89, 90, 91). Increased ketone body levels have also been observed in type 2 diabetes (92) and in congestive heart failure (93). Furthermore, in the latter condition, increased plasma ketone body concentrations correlate with the severity of cardiac dysfunction (93). The cause of the increased rate of diabetic complications in diabetic patients with frequent episodes of ketosis is not known. There is a significant relationship between the blood level of acetoacetate and in vitro oxidizability of LDL + VLDL in Type-I diabetic patients, the greater in vitro oxidizability of the LDL+ VLDL of hyperketonemic diabetic patients compared with that of normoketonic diabetic patients (94). Hyperketonemia is mainly present in Type-I diabetes, but the vascular complications of diabetes are similar in patients with Type-I and Type-II disease.
It is possible that in Type-I diabetes, acetoacetylation increases oxidative modification of LDL, which in turn increases atherogenesis (increase in oxidative modification due to the glycation of LDL). Similarly, in Type-II diabetes, elevated glucose levels alone can increase glycation and oxidative modification of LDL. This suggests that ketosis can increase the susceptibility of LDL to oxidative modification and promote the development of vascular disease in diabetes.

Studies have reported that hyperketonemic type 1 diabetic patients present increased plasma lipid peroxidation and low levels of cellular glutathione compared with normoketonemic patients (95). High levels of ketone bodies increase cellular oxidative stress, which may contribute to the development of cardiac insulin resistance in diabetes (96). They have demonstrated that prolonged exposure to β-hydroxybutyrate (OHB), the main ketone body produced during hyperketonemia (97), induces insulin resistance in cardiomyocytes (98). Ketone bodies β-hydroxybutyrate also alters metabolic stress-stimulated glucose uptake, enzyme activation of AMPK, ACC, p38 MAPK and increased oxidative stress in primary cultures of cardiomyocytes (99).

6. Subcellular defects
   6.1. Oxidative and nitrosative stress
   6.2. Proinflammatory molecules
   6.3. Impaired calcium homeostasis
   6.4. Mitochondrial dysfunction
   6.5. Alterations in stress signaling pathways
   6.6. Activation of protein kinase C isoforms

6.1. Oxidative and nitrosative stress
Oxidative and nitrosative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (100, 101). Oxidative damage is widely considered to be a cause of diabetic complications (102, 103, 104, 105), and signs of oxidative damage are found in diabetic patients. Recent proposals (106) suggest that overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be the initiating event leading to long-term development of diabetic complications.
ROS and RNS are continuously produced in most cells under physiological conditions, and their levels are regulated by a number of enzymes and physiological antioxidants. When the production of ROS exceeds the capacity of the cell to detoxify them, oxidative stress develops that is harmful to the integrity of biological tissue. Results from human and animal studies show that there is an association between oxidative damage and diabetic cardiomyopathy. Blocking ROS formation has been shown to prevent hyperglycemic induced damage (106).

6.1.1. Types of ROS and RNS generation in diabetes
Molecular species include, but by no means are limited to, superoxide (O$_2^-$), hydroxyl (·OH), peroxyl (·ROO), alkoxy radicals (·RO), and reactive molecules such as hydrogen peroxide (H$_2$O$_2$), hydrochlorous acid (HOCI) and singlet oxygen (1O$_2$), which are not free radicals but are certainly reactive and capable of causing damage (107). It is important to note that there are also reactive nitrogen species produced from similar pathways, which include the radicals nitric oxide (·NO) and nitrogen dioxide (·NO$_2$), as well as the nonradical peroxynitrite (ONOO$^-$), nitrous oxide (HNO$_2$), and alkyl peroxynitrates (RONOO$^-$). Of these free radicals play most important role in diabetic heart is yet not known.

The cellular production of one ROS may lead to the production of several others via radical chain reactions. For example, reactions between radicals and polyunsaturated fatty acids within cell membrane may result in a fatty acid peroxyl radical (R-COO·) that can attack adjacent fatty acid side chains and initiate production of other lipid radicals. Lipid radicals produced in this chain reaction accumulate in the cell membrane and may have a myriad of effects on cellular function, including leakage of the plasmolemma and dysfunction of membrane-bound receptors. End products of lipid peroxidation, including unsaturated aldehydes and other metabolites, have cytotoxic and mutagenic properties (108). Even before it was known to be NO· early studies showed that the endothelium-derived relaxing factor (EDRF) could be inactivated by O$_2^-$ and stabilized by superoxide dismutase (SOD) (109). The interaction between NO· and O$_2^-$ occurs at an extremely rapid rate (110). This is 3 times faster than the reaction rate for O$_2^-$ with SOD. Given this rapid reaction rate, there is likely always some O$_2^-$ reacting with NO· within cells and in the extracellular space and peroxynitrite (ONOO$^-$). Peroxynitrite, a very active radical similar to the hydroxyl radical,
interacts with cytoplasmic proteins to form nitrotyrosine, which has been indicated as a marker for reactive nitrogen species-induced oxidative damage under in vivo conditions (40, 42). It has been suggested that excessive NO is pathophysiological because of its ability to form pro-oxidants. Cardiac NO production and NOS protein levels have been found to be elevated in the hearts of diabetic animals (111). This is consistent with the observation of a significant increase in nitrotyrosine concentration in myocytes in the hearts of diabetic mice (42). Superoxide is probably not the only radical that can react with NO Lipid radicals (LO\(^{-}\) and LOO\(^{-}\)) can react with NO\(^{-}\) to form, respectively, LONO and LOONO (112). It is of interest that oxidized LDL, but not native LDL, added to isolated vessels inhibits endothelium-dependent vascular relaxation (113). The oxidation of LDL leads to production of linoleic hydroperoxy and alkoxy radicals that could participate in such reactions with NO\(^{-}\). Recently, it has been shown that hydroxyl radical may react with NO\(^{-}\) (114).

6.1.2. Sites of free radical generation

The sites of free radical generation encompass all cellular constituents including mitochondria, lysosomes, peroxisomes, nuclear, endoplasmic reticular, and plasma membranes as well as sites within the cytosol (Fig. 3).

![Cellular sources of free radicals generation](Fig.3. Cellular sources of free radicals generation [Adapted from Lab. Invest. 47: 412-426; 1982 (107)])
6.1.3. Sources of ROS in the heart
Direct evidence of oxidative stress in diabetic cardiomyopathy is based on studies that focused on the measurement of oxidative stress markers such as plasma and urinary F2-isoprostane as well as plasma and tissue levels of nitrotyrosine and \( \text{O}_2^- \). There could be multiple sources of oxidative stress in diabetic heart including nonenzymatic, enzymatic and mitochondrial pathways.

6.1.4. Non-enzymatic
Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate \( \cdot \text{OH} \) radicals (100). In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of \( \text{O}_2^- \).

6.1.4.1. Increased hexosamine flux and glucose autoxidation
Hyperglycemia increases flux through the hexosamine pathway by providing more fructose-6-phosphate for glutamine fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of the pathway. The effect of hyperglycemia on flux of the hexosamine pathway probably reflects increased fructose-6-phosphate levels, which result from inhibition of GAPDH by ROS (115). Recently, ROS formation due to glucose autoxidation has been hypothesized to play a role in the pathogenesis of diabetic cardiomyopathy in diabetic populations; however, no unifying hypothesis exists as to how glucose autoxidation causes any of the complications seen in diabetes.

6.1.4.2. Increased advanced glycosylation end products formation
Advanced glycosylation end-products (AGEs) are enhanced in the presence of hyperglycemia and oxidative stress (116, 117). Hyperglycemia contributes to diabetic complications through the formation of AGEs, which are irreversibly formed biochemical end products of nonenzymatic glycosylation (118). AGEs may play a key role in the pathogenesis of cardiomyopathy (119). Diabetes
produces myocardial stiffness before the development of myocardial fibrosis in association with increased formation of collagen-associated AGEs (119). AGEs can covalently crosslink and biochemically modify protein structure and affect protein functions, particularly collagen (118). They bind to their cell-surface receptors resulting in the activation of post receptor signaling, generation of intracellular ROS and the activation of gene expression (120, 121, 122). The importance of AGEs in the development of diabetic complications is seen in the observation that structurally similar dimethyl-3-phenacylthiazolium chloride, which chemically breaks AGE cross-links, led to several preclinical animal studies that showed an attenuation or reversal of disease processes of the heart retina, kidney and nervous system (123, 124, 125, 126). One of the mechanisms how AGE precursors target cells is through the binding of AGE receptors to endothelial cells, mesangial cells and macrophages, inducing receptor-mediated production of ROS. This receptor ligation increases the production of the transcription factor NF-κB, also causing increased oxidative stress. The mechanism involved in RAGE-associated development of diabetic complications appears to be related to increased production of ROS (127).

6.1.4.3. Increased polyol pathway flux

Increased glucose utilization by aldose reductase has been implicated in the development of diabetes complications. In the polyol pathway, glucose is reduced to sorbitol by aldose reductase in the presence of NADPH, and sorbitol is then oxidized by sorbitol dehydrogenase (SDH) to fructose at the cost of NAD+.

Normally, aldose reductase has a low affinity for glucose in the non-diabetic state. However the affinity for glucose is dramatically increased in the diabetic state, which converts glucose to sorbitol, with resultant decreases in NADPH. It has been demonstrated that increased aldose reductase activity in diabetic animals is correlated with increased NADH/NAD+ (128). Increasing NO availability may be a useful strategy for inhibiting the polyol pathway by aldose reductase in an inactive state and preventing the development of diabetes complications (129).

One mechanism whereby increased polyol pathway flux leads to the complications of diabetes is that oxidation of sorbitol by NAD+ increases the cytosolic NADH: NAD+ ratio, leading to inhibition of the enzyme glyceraldehydes-3-phosphate dehydrogenase (GADPH) and increased
concentrations of triose phosphate (127). Increased triose phosphate could potentially increase the formation of AGEs and diacylglycerol (DAG), thus activating PKC isoforms (127). Another mechanism whereby increased polyol pathway flux causes deleterious effects is that reduction of glucose to sorbitol by NADPH consumes NADPH, a cofactor in the generation of reduced glutathione (GSH), which could lead to increased oxidative stress. It has been shown that decreased levels of GSH are present in the lenses of transgenic mice overexpressing aldose reductase (130). These observations have led many people to believe that this is the main mechanism whereby increased polyol pathway flux leads to many of the complications seen in diabetes including cardiomyopathy.

6.1.4.4. Role of the renin-angiotensin system

The renin-angiotensin system (RAS) is known to play a major role in the regulation of blood pressure and other functions of the cardiovascular system (131). Enhanced RAS is implicated in the development of diabetic cardiomyopathy and other heart dysfunctions including coronary insufficiency, congestive heart failure and hypertensive cardiomyopathy (132). The growth-promoting effects of angiotensin II are mediated primarily through its type 1 receptor (AT1) and the action of RAS is speculated to contribute to diabetic cardiomyopathy (132). It has been shown that stimulation of the AT1 receptor generates oxygen-derived free radicals, having detrimental effects on the cardiovascular system (133, 134). The AT1 receptor has been shown to be coupled to several post receptor signaling pathways, including Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and NADPH oxidase (131, 135, 136). However, the precise role of RAS, in particular the AT1 receptor, in the development of diabetic cardiomyopathy is still speculative.

6.1.4.5. Mitochondrial pathway

The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and FADH₂, through four complexes in the inner mitochondrial membrane to oxygen, generating ATP in the process (137). Under normal conditions, O₂⁻ is immediately eliminated by natural defense mechanisms. A recent study demonstrated that hyperglycemia-induced generation
of $O_2^-$ at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (106, 127).

Increased mitochondrial ROS generation has been shown in various tissues such as endothelial cells that are exposed to hyperglycemia (138). Relatively few studies to date have directly measured mitochondrial ROS production in mitochondria obtained from diabetic hearts. However, overexpression of mitochondrial superoxide dismutase (Mn-SOD) in the heart of a mouse model of type 1 diabetes mellitus reversed altered mitochondrial morphology and function and maintained cardiomyocyte function (139).

### 6.1.5. Enzymatic

Enzymatic sources of augmented generation of reactive species in the diabetic heart could include NAD(P)H oxidase (NOX), Xanthine dehydrogenase and oxidases (XO), cyclooxygenase (COX-2), lipoxygenases (LO), monoamine oxidases-A, (MAO-A) and nitric oxide synthase (NOS). All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH$_4$ and Ca$^{2+}$-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce $O_2^-$ instead of •NO and this is referred to as the uncoupled state of NOS (101, 140, 141, 142).

#### 6.1.5.1. NADH/NADPH Oxidase

Of the various enzymatic sources of ROS in the heart suggested, NADPH oxidases seems to have emerged as major ROS sources in the heart. Guzik et al (140) reported that both diabetes and hypercholesterolemia are associated with increased NADH-dependent $O_2^-$ production. There remain several questions about the NADH/NADPH oxidases of vascular tissues. The subunits of these enzymes have not been identified precisely, and how they interact is not understood. The NADPH oxidases are a group of plasma membrane-associated enzymes found and characterized not only in neutrophils but also in endothelial cells, vascular smooth muscle cells, and cardiac myocytes (131, 143, 144, 145, 146). The prototypic NADPH oxidase comprises a membrane-bound p22$^{phox}$/gp91$^{phox}$ heterodimer and 4 regulatory subunits, p40$^{phox}$, p47$^{phox}$, p67$^{phox}$, and rac1 (147). Several gp91$^{phox}$ homologues (termed Noxs) have recently been
identified (148), however, a gp91$^{\text{phox}}$ (or Nox2) containing oxidase is known to be expressed in endothelium, fibroblasts, and cardiomyocytes (143, 144).

The molecular events at the level of the oxidase that are involved in its acute activation in cardiovascular cells are best characterized for the classical Nox2 containing oxidase and Nox1. In general, Nox2 oxidase activation in these cells involves a similar process to that in neutrophils, namely the association of cytosolic oxidase components (p47$^{\text{phox}}$, p67$^{\text{phox}}$ and Rac1) with cytochrome b$_{558}$. Binding of p67$^{\text{phox}}$ to an activation site on Nox2 initiates the electron transfer process but the key post-translational modifications involved in oxidase activation are the phosphorylation of p47$^{\text{phox}}$ and Rac activation (149). NADPH oxidase catalyzes the production of superoxide (2O$_2^-$) by the one-electron reduction of oxygen, using NADPH as the electron donor (143, 147) (Fig. 4).

\[
2\text{O}_2 + \text{NADPH} \rightarrow 2\text{O}_2^- + \text{NADP}^+ + \text{H}^+
\]

The enhanced expression of the NADPH oxidase subunits results in the increase of ROS in diabetic rat hearts. An increase in the cardiac protein expression of p67$^{\text{phox}}$ and p22$^{\text{phox}}$ accompanied by increased NADPH-dependent superoxide production has been shown to occur in diabetic rats (490). Additional studies have shown that the mRNA expression of p22$^{\text{phox}}$ is increased in angiotensin II–induced hypertension (223). Accumulating evidence suggests that the NADH/ NADPH oxidase may be responsible for excessive O$_2^-$.

**Fig. 4: Structure and activation of Nox2 NADPH oxidase:** Activation involves translocation of the cytosolic subunits p47$^{\text{phox}}$, p67$^{\text{phox}}$, p40$^{\text{phox}}$ and Rac to the membrane where they bind to cytochrome b$_{558}$, composed of p22$^{\text{phox}}$ and gp91$^{\text{phox}}$ (Nox2) subunits [Adapted from Biochemical Society Transactions. 2006; 34:960-964 (150)].
However, although NADPH oxidase has been shown as a major source of ROS in diabetic cardiomyopathy the relative importance of other oxidases such as XO, MAO-A, COX-2 and 5-LO has not been studied.

6.1.5.2. Xanthine oxidase
Another source of ROS is xanthine oxidoreductase. It is a molybdoenzyme capable of catalyzing the oxidation of hypoxanthine and xanthine in the process of purine metabolism. Xanthine oxidoreductase can exist in two inter convertible forms, either as xanthine dehydrogenase or xanthine oxidase. The former reduces NAD\(^+\), whereas the latter prefers molecular oxygen, leading to the production of both O\(_2^-\) and H\(_2\)O\(_2\). Xanthine oxidase catalyzes the oxidation of xanthine and hypoxanthine into uric acid, producing O\(_2^-\) and H\(_2\)O\(_2\) as a by-product (151) (Fig. 5).

**Fig. 5: Metabolism of xanthine by xanthine dehydrogenase:** Scheme showing oxidative hydroxylation of hypoxanthine to xanthine to uric acid and generate free radicals by xanthine dehydrogenase

In endothelial cells, the activity and expression of xanthine oxidase is enhanced by IFN-\(\gamma\) (152). The first suggestion that O\(_2^-\) derived from xanthine oxidase might alter NO\(_2\) bioavailability came from studies of spontaneously hypertensive rats (SHRs). In these animals, a recombinant form of SOD modified to bind to heparin-binding sites dramatically lowered blood pressure but had no effect on blood pressure in non-hypertensive rats. In these same animals, the xanthine oxidase inhibitor oxypurinol also lowered blood pressure, strongly suggesting that xanthine oxidase played a role in this process (153). There is also evidence that free radical production is increased in the microcirculation of SHRs and that this can be prevented by a xanthine oxidase inhibitor (154). Previous studies have shown that early stages of experimental atherosclerosis caused by diet induced hypercholesterolemia are associated with increased O\(_2^-\), presumably from
xanthine oxidase, because \( \text{O}_2^- \) production normalized by oxypurinol (155). In humans with hypercholesterolemia, administration of oxypurinol inhibits \( \text{O}_2^- \) production, improved impaired vasodilation in hypercholesterolemic patients (156).

### 6.1.5.3. Endothelial nitric oxide synthase

Other source of ROS production that has received substantial attention is endothelial nitric oxide synthase (eNOS). That catalyzes flavin-mediated electron transport from the electron donor NADPH to a prosthetic heme group. The enzyme requires tetrahydrobiopterin (BH4), bound near this heme group, to transfer electrons to guanidine nitrogen of L-arginine to form nitric oxide. In the absence of either L-arginine or BH4, eNOS can produce \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \). This phenomenon has been referred to as NOS uncoupling (157, 158, 159). There has also been evidence presented that eNOS can become uncoupled in vivo in a variety of pathophysiological conditions. In the aorta of stroke-prone spontaneously hypertensive rat, \( \text{O}_2^- \) production is increased, and this can be normalized by treatment with L-NAME or removal of the endothelium (160). Impaired endothelium-dependent vasorelaxation has been observed in rats made insulin resistant by high-fructose feeding and has been normalized by supplement with BH4 (161). Intra-arterial infusion of BH4 has been shown to improve endothelium-dependent vasodilation in chronic smokers, suggesting that depletion of BH4 may have impact on turning eNOS into \( \text{O}_2^- \) generating enzyme in human (162). Uncoupling of eNOS in the endothelium may lead to oxidative stress and endothelial dysfunction via enzymatic production of NO’ is diminished; allowing the radicals that it normally might react with to attack other cellular targets. And the enzyme begins to produce \( \text{O}_2^- \), contributing to oxidative stress. Finally, it is likely that eNOS can become partially uncoupled, such that both \( \text{O}_2^- \) and NO’ are produced simultaneously. Under this circumstance, eNOS may become a peroxynitrite generator, leading to a dramatic increase in oxidative stress.

### 6.1.5.4. Monoamine oxidase-A

Serotonin (5-HT) is a biogenic amine produced in the central nervous system by cells originating in the raphe nuclei of the brainstem. In periphery, 5-HT is produced predominantly by intestinal enterochromaffin cells and a large amount
of 5-HT is stored in platelets and released during platelet activation. 5-HT affects a wide variety of physiological functions through the interaction with specific G-coupled membrane receptors. In the heart, 5-HT has been involved in regulation of normal cardiac development (163) and in different diseases, including arrhythmia (164), ventricular hypertrophy (165). During the last years, several studies showed that serotonin accumulates in heart during ischemia-reperfusion (I/R) and contributes to the progression of myocardial injury and dysfunction (166, 167). The deleterious effects of serotonin have been related to indirect mechanisms involving coronary vasoconstriction (168) and reactive oxygen species (ROS) dependent stimulation of cardiac sympathetic afferents (166, 169).

The outer mitochondrial membrane enzymes monoamine oxidase (MAO) catalyzes the oxidative deamination of neuroactive, vasoactive (serotonin, dopamine, catecholamines), or dietary (tyramine) amines and xenobiotics, releasing reactive aldehydes and hydrogen peroxide (H$_2$O$_2$) (170). Two isoforms of the enzyme, MAO-A and MAO-B, which are encoded by two different genes and are distinguished by their differential affinity for substrates, sensitivity to inhibitors, and tissue distribution have been identified (171). MAO-A preferentially oxidizes norepinephrine and serotonin and is selectively inhibited by clorgyline, whereas MAO-B oxidizes β-phenylethylamine and benzylamine and is inhibited by pargyline and deprenyl. Both enzymes oxidize dopamine and tyramine (171, 172). Recent findings show that the serotonin-degrading mitochondrial enzyme monoamine oxidase A (MAO-A) is an important source of hydrogen peroxide (H$_2$O$_2$) in the heart (173). In the heart, MAO-A is a predominant enzyme involved in the deamination of endogenous or exogenous amines (174). The predominant form of MAO found in the heart is MAO-A (173, 175). Recently oxidative stress induced by MAO-A has been shown to be responsible for receptor-independent, serotonin-mediated apoptosis during post ischemic myocardial injury (176, 177). However, the role of MAO in diabetic cardiomyopathy is yet not known.

6.2. Proinflammatory molecules

6.2.1. Alteration of cytokines profile

Cytokines are group of proteins and peptides that are signalling compounds produced by animal cells to communicate with one another. They act by binding
to specific cell-surface cytokine receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its behavior (gene expression). Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory, and infectious diseases. Subsequent cascades of intracellular signaling then alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcription factors, in turn resulting in the production of other cytokines.

Recent investigations suggest that diabetic conditions induce inflammatory responses by oxidative mechanisms, which might contribute to the development of diabetic cardiomyopathy (178, 179, 180). The possible involvement of intramyocardial inflammation in diabetic cardiomyopathy, CAMs expression (ICAM-1 and VCAM-1) and β2-leukocyte-integrins infiltrates, as well as cytokine (IL-1β and TNF-α) expression in STZ-induced diabetic rats (181). Multiple pathogenic mechanisms are responsible for the endothelial CAMs induction, such as proinflammatory cytokine expression, oxidative stress, and hyperglycemia (182, 183, 184). Endothelial abundance of cell adhesion molecules (CAMs) is associated with endothelial dysfunction in human-dilated cardiomyopathy, implicating a functional role of inflammatory endothelial activation in heart failure (185, 186). Intra-myocardial inflammation, characterized by immunocompetent infiltrates and induction of endothelial CAMs, has been confirmed in a significant proportion of dilated cardiomyopathy patients (185).

In recent years, it has been firmly established that inflammation not only contributes to the initiation and progression of atherosclerosis but is also a key player in the cardiac outcome of acute coronary syndromes (187). However, little is known about the potentially unique features of this inflammatory process in diabetes. Several inflammatory markers have been associated with cardiovascular events, including cytokines and growth factors, which are released by activated macrophages (188). Compared with nondiabetic mice, both iNOS+/+ and iNOS−/− diabetic mice had higher tissue levels of cytokines implicated in plaque destabilization (IL-18) and future cardiovascular events (IL-6 and TNF-α). Acute hyperglycemia in healthy subjects and in patients with impaired glucose tolerance increases the circulating levels of these cytokines (188), which are also able to stimulate the production of iNOS by mononuclear and mesenchymal cells (189). Hyperglycemia induced oxidative stress (190), along with soluble advanced
glycation end products and products of lipid peroxidation, possibly serves as a key activator of upstream kinases, leading to induction of inflammatory gene expression (191).

Diverse pathogenic mechanisms contribute to diabetic cardiomyopathy, including changes in the composition of the extracellular matrix with enhanced cardiac fibrosis and increased cardiac cytokine levels (192, 193). These pathological mechanisms are mediated by hyperglycemia (194) and by increased aldosterone and angiotensin II (ANGII) levels (195). Cardiac cytokines that are stimulated by ANGII, such as interleukin IL-1β and transforming growth factor TGF-β1, are involved in the development of cardiac fibrosis and cardiac failure (196, 197, 198).

Cytokines can attenuate myocyte contractility directly through alterations in sarcoplasmic reticulum function and indirectly by down regulating the sarcoplasmic calcium ATPase expression (199). Moreover, they are also known to regulate the extracellular matrix. TGF-β acts as a profibrotic growth factor by upregulating the connective tissue growth factor through gene transcription (200). TGF-β itself is upregulated by ANGII via the AT-1 receptor, as shown in obstructive nephropathy (201). IL1-β is known to be an important mediator in inflammatory diseases by stimulating lymphocytes as well as bone marrow cells (202). Pomenrantz et al. (203) showed a direct cardio depressive effect of IL-18 after ischemia and reperfusion injury in human atrial myocardium. They conclude that not only the proinflammatory cytokines IL1-β and TNF-α, but also IL-18 are responsible for cardiac dysfunction (204).

6.2.2. 5-Lipoxygenase

Although arachidonic acid is known to exert direct functional effects in vitro, it is also further metabolized by cyclooxygenase (COX) enzymes to produce prostaglandins (205) and lipoxygenases (LO) to produce hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (206). Depending on the oxygenation site in arachidonic acid, the LO enzymes are termed 5-, 12-, and 15-LO. The roles played by LO enzymes in diabetic cardiomyopathy have not been fully established, but there is evidence that 12-LO (207, 208) play roles in cytokine-mediated damage of β-cells. There are also some contradictory reports about the effects of COX and LO products on insulin secretion (209, 210, 211, 212).
5-lipoxygenase involved in the production of leukotrienes and ROS from arachidonic acid (213), overexpression of 5LO promotes growth arrest via a p53/p21 dependent pathway, and this is regulating by ROS production. Increased production of ROS may derive from the activation of NADPH oxidase and from mitochondrial disruption. There are other sources of cellular ROS, including the signaling of arachidonic acid, which catalyze by 5-LO. The activity of 5-LO is required for ROS production by CD28 stimulation in T lymphocytes and production of 5-LO cascade. Both 5-LO and COX2 are oxidizing enzymes forming hydroperoxy fatty acids, and lipids peroxides may be involved in the generating of ROS and stasis. Type of ROS formed in 5-LO expressing cell is depends upon the type of cell it produced superoxide, H$_2$O$_2$, NO', and lipid per oxidation.

**6.2.3. Cyclooxygenase-2**

In the Cyclooxygenase (COX) pathway, arachidonic acid is converted to prostaglandin H2 by COX-1, which is constitutively expressed in the most tissues, and by COX-2, which is an inducible isoform (214) which is induced in response to growth factors, cytokines, phorbol esters, and lipopolysaccharide (LPS) (215, 216, 217, 218). Prostaglandin H2 is further converted to prostacyclin (PGI2) by PGI2 synthase (PGIS), the main prostanoid produced in the vascular endothelium of many species, including humans (219). In addition, COX-1, COX-2, and PGIS are also present in vascular smooth muscle cells, including human coronary arteries (220, 221). Taylor (222) reported impaired PGI2-induced vasorelaxation in the mesentery artery of type 1 diabetes. In type 2 diabetic patients with angiopathy, serum PGI2 levels are decreased (223). The impaired endothelial dysfunction in diabetic rats can be restored by oral administration of a PGI2 analog (224).

Cyclooxygenase-2 (COX-2) mediated prostaglandin production by activated macrophages is associated with inflammation and atherosclerosis. It is now well established that vascular inflammation plays a role in the pathogenesis of atherosclerosis (225). Activated macrophages are a key component not only in the development of atherosclerosis but also in plaque rupture leading to thrombosis (226). Several studies have demonstrated the presence of COX-2 in atherosclerotic plaques, mainly co-localizing with macrophages (227, 228, 229).
In addition, it has been shown that there is increased co-expression of COX-2 and matrix metalloproteinases (MMPs), enzymes that are involved in the destabilization of atherosclerotic plaques (230). Furthermore, selective inhibition of COX-2, with rofecoxib, resulted in a significant reduction in aortic atherosclerosis in low density lipoprotein (LDL) receptor-deficient mice (231). PGE2 production by stimulated monocytes was related to a clustering of cardiovascular risk factors in a population sample of adults free from clinically overt cardiovascular disease. PGE2 levels increased with increasing number of cardiovascular risk factors, mainly smoking and diabetes, (232, 233).

6.3. Impaired calcium homeostasis

Intracellular calcium ($Ca^{2+}$) is a major regulator of cardiac contractility. In the cardiomyocyte, $Ca^{2+}$ influx induced by activation of voltage-dependent L-type $Ca^{2+}$ channels on membrane depolarization triggers the release of $Ca^{2+}$ via $Ca^{2+}$ release channels (ryanodine receptors) of sarcoplasmic reticulum (SR). It has long been appreciated that calcium and other ion homeostasis is altered in diabetic cardiomyocytes (234). The mechanisms by which disturbed calcium homeostasis alters cardiac function in diabetes include reduced activity of ATPases (235), decreased ability of the SR to take up calcium, and reduced activities of other exchangers such as $Na^{+}$-$Ca^{2+}$ and the sarcolemmal $Ca^{2+}$ ATPase (236, 237). Indeed, the SR $Ca^{2+}$ store and rates of $Ca^{2+}$ release and reuptake into SR were depressed in type 1 diabetic rat myocytes. The rate of $Ca^{2+}$ efflux via sarcoplasmal $Na^{+}$-$Ca^{2+}$ exchanger also was depressed. In the db/db mouse model of type 2 diabetes mellitus, $Ca^{2+}$ efflux in the cardiomyocyte was reduced, SR $Ca^{2+}$ load was depressed, ryanodine receptor expression was reduced, and $Ca^{2+}$ efflux through the $Na^{+}$-$Ca^{2+}$ exchanger was increased (238). Furthermore, decreased cardiac expression of SERCA2a or the $Na^{+}$-$Ca^{2+}$ exchanger has been observed in type 1 (239) and type 2 diabetes (240). Trost et al (241) observed that transgenic mice overexpressing SERCA2a were protected from streptozotocin-induced cardiac dysfunction, suggesting that altered calcium handling contributes to impaired cardiac function in diabetes mellitus. Recent study in human described depressed myofilament function as a result of decreased $Ca^{2+}$ sensitivity in skinned fibers obtained from diabetic patients at the time of coronary artery bypass surgery (242).
6.4. Mitochondrial dysfunction

Recent studies of mitochondria have reignited interest in a role for mitochondrial dysfunction in the pathogenesis of diabetic cardiomyopathy (243, 244, 245). Diabetes mellitus causes functional and structural alterations in mitochondria. Impaired mitochondrial function was initially reported almost 25 years ago when Kuo et al. (246) showed depressed respirations in db/db heart mitochondria. This study was followed by others showing reduced mitochondrial oxidative capacity in type 1 diabetes (246, 248). Decreased into mitochondrial respiration and reduced protein expression of the oxidative phosphorylation components in obese type 2 diabetic mice (249). These alterations contribute to cardiac dysfunction because they reduce ATP production, thereby contributing to impaired myocardial contractility. In addition to reduced oxidative phosphorylation capacity, mitochondria from hearts of type 1 diabetic animals exhibit a lower creatine phosphate activity (250, 251) lower ATP synthase activity (247), and lower creatine-stimulated respiration (252). Additional mitochondrial defects include decreased mitochondrial calcium uptake (248) attributed in part to enhanced permeability transition (253). Mitochondrial dysfunction in diabetes may reflect in part transcriptional repression of genes involved in oxidative phosphorylation components. Thus, a reduction in mRNA levels of cytochrome b and ATP synthase subunit 6 (mitochondria-encoded genes) was found in streptozotocin-induced diabetic hearts (254, 255). This was associated with a reduction in the binding capacity of mitochondrial transcription factor A to mitochondrial DNA. In addition, proteomic analysis of the diabetic heart also revealed reduced expression of proteins of the electron transport chain, creatine kinase, and the voltage-dependent anion channel 1, whereas the expression of β-oxidation proteins was increased (256). These changes could lead to mitochondrial dysfunction in a number of ways. Increased β-oxidation will increase the delivery of reducing equivalents to the electron transport chain. However, a limitation in oxidative phosphorylation components might result in increased superoxide production, which in turn might uncouple mitochondria and reduce the efficiency of ATP generation, which compounds existing defects in respiratory capacity (243, 249). Indeed, perfusion of ob/ob hearts with FAs clearly increased mitochondrial oxygen consumption in mitochondria from ob/ob mice that was associated with a
further reduction in ATP generation, indicating mitochondrial uncoupling (249). Mitochondrial protein nitration (as an index of oxidative damage) was increased in hearts from alloxan-induced diabetes (256). Mitochondrial hydrogen peroxide production was increased and glutathione levels were reduced in diabetic hearts, and these changes were attenuated by rotenone, thereby suggesting a mitochondrial source for ROS (254). Overexpression of ROS detoxifying proteins (metallothionein, catalase, and manganese superoxide dismutase) reverses mitochondrial dysfunction and cardiomyopathy induced by diabetes (139, 257, 258). Recent studies in humans have provided support for a role of mitochondrial dysfunction in diabetic cardiomyopathy. Reduction in the ratio of phosphocreatine to ATP has been observed in patients with type 1 and type 2 diabetes mellitus without clinically significant coronary artery disease and correlates with indexes of diastolic dysfunction and with levels of serum free FAs (259, 260). Ultra-structural analyses have revealed increased mitochondrial proliferation in models of type 1 (261) and type 2 diabetes Mellitus (249) and even in mouse models of the metabolic Syndrome (262). Gene knockouts of critical mitochondrial proteins such as the adenine nucleotide translocase and mitochondrial transcription factor A lead to increased mitochondrial biogenesis and cardiac myopathies (263, 264).

6.5. Alterations in stress signaling pathways
Free radicals can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications. \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) stimulate stress-related signaling mechanisms such as NF-\( \kappa \)B, p38-MAPK and STAT-JAK resulting in diastolic dysfunction. Blocking ROS and superoxide formation, however, has been shown to prevent hyperglycemia-induced organ damage in diabetes (106). One major intracellular target of hyperglycemia and oxidative stress is NF-\( \kappa \)B (265, 266, 267) which can be activated by a variety of exogenous and endogenous stimuli, including hyperglycemia, elevated free fatty acids, ROS, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and other proinflammatory cytokines, p38 MAP kinase and ultraviolet irradiation (267). Alterations in NF-\( \kappa \)B signaling are associated with a number of chronic
diseases, such as diabetes and atherosclerosis. The c-jun NH(2)-terminal kinases (JNK) and p38 MAPKs are members of the complex super family of MAP serine/threonine protein kinases and are known as stress-activated kinases. Activities of these enzymes are stimulated by a variety of exogenous and endogenous stress-inducing stimuli, including oxidative stress, ROS, hyperglycemia and proinflammatory cytokines (39, 79, 268, 269). Taken together, these suggest that NF-κB, JNK and p38 MAPK pathways are potential stress-signaling systems that can lead to late complications of diabetes (Fig. 6). Several signal transduction pathways lead to alterations in gene expression, while others might lead to the modulation of enzyme activities. JAK/STAT, Janus kinase/signal transducers and activators of transcription.

![Diagram](image.png)

**Fig. 6: Potential intracellular signalling pathways that might be acted upon by hydrogen peroxide.** Several signal transduction pathways leads to alterations in gene expression, while others might lead to modulation of enzymatic activity [(Adapted from Biochem. Soc. Trans. 2001; 29:345-50(270)]

6.6. **Activation of protein kinase C isoforms**

Protein kinase C is also increased in the tissues of diabetic patients (271). Activation of the PKC pathway by hyperglycemia can occur directly or indirectly via ligation of AGE receptors (272) or increased activity of the polyol pathway (273) and can synergize with other kinase pathways, that is, the MAPK pathway. Interactions between these pathways are likely to play a role in determining the
long-term effects of hyperglycemia. Sorbitol, whose formation from glucose is catalyzed by aldose reductase, is increased when intracellular glucose concentrations rise (274), and can accumulate intracellularly which can cause cell damage. The p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) pathways are also activated by sorbitol. The significance of the sorbitol pathway as a cause of diabetic complications was demonstrated in transgenic mice that overexpressed the aldose reductase gene (130, 275, 276) and inhibitors of this enzyme prevent the development of long-term diabetic complications in these animals (277).

7. Cellular targets and consequences of free radical damage

Excess generation in oxidative & nitrosative stress has pathological consequences including damage to proteins, lipids and DNA. Prime targets for free radical reactions are the unsaturated bonds in membrane lipids. Consequent peroxidation results in a loss in membrane fluidity and receptor alignment and potentially in cellular lysis. Free radical damage to sulfur-containing enzymes and other proteins culminates in inactivation, cross-linking, and denaturation. Nucleic acids can be attacked and subsequent damage to the DNA can cause apoptosis leading to contractile dysfunction and cardiomyopathy (Fig. 2, 7).

Oxidative damage to carbohydrates can alter any of the cellular receptor functions including those associated with insulin responses. Free radicals such as the superoxide anion, hydroxyl radical, peroxyl radicals and peroxynitrite are responsible for many of the damaging reactions. In addition, certain aldehydes such as malondialdehyde and hydroxynonenal, arising from the free radical degradation of polyunsaturated fatty acids, can cause cross-linking’s in lipids, proteins, and nucleic acids (107, 279, 280, 281). ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (282).
Fig. 7: Proposed role of oxidative/nitrosative stress and downstream pathway in heart failure. Peroxynitrite, formed from superoxide and NO, causes cell injury via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration and also activation of matrix metalloproteinases (MMPs) just to mention a few. Peroxynitrite also triggers the release of proapoptotic factors such as cytochrome c and apoptosis inducing factor (AIF) from the mitochondria, which mediate caspase dependent and independent apoptotic death pathways. Peroxynitrite together with other oxidants induces stand breaks in deoxyribonucleic acid (DNA), which in turn activates PARP. Over activated PARP initiates an energy consuming cycle by transferring adenosine diphosphate-ribose units from nicotinamide adenine dinucleotide (NAD+) to nuclear proteins. This process will lead to depletion of the intracellular NAD+ and adenosine triphosphate (ATP) pools, slowing the rate of glycolysis and mitochondrial respiration, eventually culminating to cardiovascular dysfunction or death. Poly (ADP-ribose) glycohydrolase (PARG) degrades poly (ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose. PARP also regulates the expression of a variety of inflammatory mediators, which may facilitate the progression of heart failure [Adapted from Current Vascular Pharmacology, 2005, 3, 221-229 (278)].
8. Cellular protection against oxidative damage

Many enzymic and non-enzymic mechanisms are present in cells to achieve cellular protection against oxidative damage (283). The enzymatic defense system included several metalloenzymes including glutathione peroxidase (selenium), catalase (iron), and superoxide dismutase (copper, zinc, manganese). Superoxide dismutase (SOD) catalyzes the conversion of $\text{O}_2^-$ to $\text{H}_2\text{O}_2$ and molecular oxygen (284). The cytosolic form of SOD contains Cu and Zn (Cu/Zn-SOD), while a mitochondrial form contains Mn (Mn-SOD) (285). Glutathione peroxidase (GSH-Px) and catalase detoxify hydrogen peroxide ($\text{H}_2\text{O}_2$) in the mitochondria and lysosomes, respectively (286, 287). $\text{H}_2\text{O}_2$ can also be converted to the highly reactive $'\text{OH}$ radical in the presence of transition elements like iron and copper. Heat shock protein hemeoxygenase (HO-1) (288) metabolizes the prooxidant heme to the antioxidant biliverdin, carbon monoxide, and free iron. Biliverdin is reduced to another antioxidant, bilirubin, by biliverdin reductase (288, 289, 290). However, besides enzymes, many dietary components have antioxidant capacity, including $\alpha$-tocopherol (vitamin E), ascorbic acid (vitamin C), $\beta$-carotene, glutathione, uric acid, bilirubin, and proteins such as ceruloplasmin (copper).

The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system. The heart is a susceptible target because it contains low levels of free radical scavengers or antioxidants (291) therefore, more susceptible to oxidative and nitrosative stress. Several dietary micronutrients contribute greatly to the protective system. Based on the growing evidence of oxidative stress in diabetic cardiomyopathy and the lack of effective therapies, the usefulness of essential, safe nutrients in protecting against the adverse effects of oxidative injury warrants further study. Many of the protective antioxidants are essential nutrients or have essential nutrients as part of their molecule. In the field of nutrition, the term essential is given to those nutrients (like the vitamins) that must be consumed.

9. Essential nutrients with antioxidant functions

Only three essential nutrients can directly scavenge free radicals. Vitamin E ($\alpha$-tocopherol), the major lipid soluble antioxidant present in all cellular membranes, protects against lipid peroxidation (280). It can act directly with a variety of oxy
radicals, including the peroxo radical (ROO•), CCl₃ and HO₉. (292, 293), superoxide radical (294) and with singlet oxygen (295). Vitamin C (ascorbic acid) is water soluble and, along with vitamin E, can quench free radicals as well as singlet oxygen. Ascorbic acid has been shown to react directly with superoxide (296), hydroxyl radicals (297), and singlet oxygen (298). Ascorbic acid can also regenerate the reduced, antioxidant form of vitamin E. In the presence of transition metals, ascorbic acid can provoke the formation of free radicals (Fig. 8). It is quite clear that vitamin E does function as an antioxidant in vivo as evidenced by the increased concentrations of aldehydes, peroxides, and lipofuscin in the tissues of vitamin E-deficient animals. Significantly increased levels of ethane and pentane in the exhaled air of vitamin E-deficient rats as well as from vitamin C deficient guinea pigs provide further evidence of increased lipid peroxidation when these nutrients are absent from the diet (299, 300). Recent work has shown that β-carotene is the most efficient quencher of singlet oxygen known in nature and can also function as an antioxidant (301). β-Carotene is the major carotenoid precursor of vitamin A. Vitamin A, however, cannot quench singlet oxygen and has a very small capacity to scavenge free radicals (302, 303). These three nutrients are also present in relatively high concentrations in the serum. There are, in addition, several nutritionally essential minerals incorporated into protective antioxidant enzymes. Zinc, copper, and manganese are required for the activity of the two types of superoxide dismutases. Selenium, an essential component of glutathione peroxidase, is important in the decomposition of hydrogen peroxide and lipid peroxides. The level of dietary intake of all the antioxidant micronutrients directly affects the circulating level of these nutrients and the activity of the antioxidant metalloenzymes. Thus, low intakes of one or more of these antioxidant nutrients could reduce the body’s defenses against free radical damage and increase susceptibility to health problems associated with free radical damage.

Therefore, when considering how far ROS will travel, or in which part of the cell ROS will act, one has to consider that cells and organelles alike are well protected from the presence of ROS by a variety of means.
Fig. 8: The sources and types of ROS and RNS in the diabetic heart, compartmentalized in cytosol and mitochondria. Cellular injury leads to DNA damage, lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration. Multiple antioxidants, a mixture of nonenzymatic and enzymatic antioxidants supplemented with diet are powerful weapons that may act together and minimize oxidative and nitrosative stress by inhibiting ROS and RNS formation inside the cell and protect the heart.

10. Antioxidant interactions

In addition to direct quenching of reactive, damaging free radicals, vitamin C has been clearly shown to interact with the tocopheroxyl radical and to regenerate the reduced tocopherol. The evidence for this important antioxidant function for ascorbic acid includes cell-free experiments, investigations with liposomal membranes, and recently, in vivo evidence of higher concentrations of vitamin E in tissues of guinea pigs fed high dietary levels of vitamin C (299, 300). Vitamin E can protect the conjugated double bonds of β-carotene from oxidation. The
sparing action of tocopherol on β-carotene was first described in vivo in humans by Urbach et al. (302). Vitamin E can protect against many of the symptoms of selenium deficiency and vice versa (304, 305). These sparing as well as synergistic actions are thought to result from the ability of both tocopherol and selenium-dependent glutathione peroxidase to decrease the production of lipid peroxidation products. Vitamins C and β-carotene Glutathione Peroxidase Keeping this in mind we have planned our experiments using multiple antioxidants that can scavenge or remove multiple oxidants.

11. Role of antioxidants in diabetic cardiomyopathy

Through the respiratory chain, a series of oxidation-reduction reactions continually take place in the myocyte. Therefore, an efficient antioxidant system, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione (GSH) and α-tocopherol are critical to effective functioning of the myocardium (Fig. 8). However, in experimental animal models, the heart levels of these antioxidants are much lower in compare to other organ systems, even in non-diabetic (291, 306). In addition, hyperglycemia can impair and decrease the amount of antioxidants within the heart of a diabetic animal (307, 308, 309) making it more vulnerable to ROS-induced damage. The increase in ROS serves to decrease the antioxidant capacity of the diabetic myocardium, contributing significantly to oxidative stress and resultant myocardial damage. This damage causes cardiac morphological and functional abnormalities. Epstein and colleagues (258, 287) showed that type 1 diabetic cardiomyopathy could be prevented when the antioxidants metallothionein (MT) and catalase were over expressed specifically in the heart. They also showed that ROS production was enhanced in genetically diabetic mice (OVE26), which could be prevented by genetically crossing the diabetic mice with those overexpressing the MT or catalase genes (258, 287).

Mitochondrial damage is related to ROS formation and plays an important role in the development of diabetic cardiomyopathy (310, 311). Coenzyme Q (CoQ) is an important component in mitochondrial energy metabolism and is also a potent endogenous antioxidant in vivo. In heart mitochondrial preparations of diabetic rats, the concentration of α-tocopherol was increased; however, the concentration of both CoQ-9 and CoQ-10 was decreased (311). The reduction in coenzyme
levels from diabetic animals is attenuated with the supplementation of insulin-like growth factor I (IGF-1) (312). Heart specimens from diabetic patients showed an increase in myocyte, endothelial and fibroblast apoptosis (40). The increased cell death was associated with an increase in ROS formation (40, 41, 42). However, the precise mechanism by which ROS accumulation leads to compromised heart function and the effect of antioxidant therapy in diabetic subjects is largely unknown.

![Diagram](image)

**Fig. 9:** *Scheme of possible mechanisms by which hyperglycemia induces ROS and RNS and the subsequent effects leading to cardiomyopathy [(Adapted from Cardiovascular Toxicology. 2001; 1: 181-93(30)]*

### 12. Treatments for diabetes

#### 12.1. Pharmaceutical antidiabetic drugs

Treatment of type I diabetes is limited to insulin replacement, while type II diabetes is treatable by a number of therapeutic approaches. Many cases of insulin resistance are asymptomatic due to normal increases in insulin secretion, and others may be controlled by diet and exercise. Drug therapy may be directed
toward increasing insulin secretion, increasing insulin sensitivity, or increasing insulin penetration of the cells.

Pharmaceutical antidiabetic drugs may be subdivided into six groups: insulin, sulfonylureas, α-glucosidase inhibitors, biguanides, meglitinides, and thiazolidinediones. Insulin (Humulin, Novolin) is effective in both types of diabetes, since, even in insulin resistance, some sensitivity remains and the condition can be treated with larger doses of insulin. Isophane insulin suspension, insulin zinc suspension, and other formulations are intended to extend the duration of insulin action, and permit glucose control over longer periods of time. Sulfonylureas (chlorpropamide, tolazamide, glipizide) act by increasing insulin release from the β-cells of the pancreas. Glimepiride, a member of this class, appears to have a useful secondary action in increasing insulin sensitivity in peripheral cells. α-glucosidase inhibitors (acarbose, miglitol) do not enhance insulin secretion. Rather, they inhibit the conversion of disaccharides and complex carbohydrates to glucose. This mechanism does not prevent conversion, but only delays it, reducing the peak blood glucose levels. Metformin is the only available member of the biguanide class. Metformin decreases hepatic (liver) glucose production, decreases intestinal absorption of glucose and increases peripheral glucose uptake and use. Metformin may be used as monotherapy, or in combination therapy with a sulfonylurea. The mechanism of action of the meglitinides is to stimulate insulin production. This activity is both doses dependent and dependent on the presence of glucose, so that the drugs have reduced effectiveness in the presence of low blood glucose levels. Rosiglitazone and pioglitazone are members of the thiazolidinedione class. They act by both reducing glucose production in the liver, and increasing insulin dependent glucose uptake in muscle cells. They do not increase insulin production

Despite the availability of a variety of pharmaceutical drugs, they involve numerous side effects and the treatment with them should involve utmost precaution. The greatest short term risk of insulin is hypoglycemia, which may be the result of either a direct overdose or an imbalance between insulin injection and level of exercise and diet. This may also occur in the presence of other conditions which reduce the glucose load, such as illness with vomiting and diarrhea. Allergic reactions and skin reactions may also occur. Insulin glargine (Lantus), an insulin analog which is suitable for once-daily dosing, there have been reported
changes in the hearts of newborns in animal studies of this drug. Insulin is not recommended during breast feeding because either low of high doses of insulin may inhibit milk production. All sulfonylurea drugs may cause hypoglycemia. Most patients become resistant to these drugs over time, and may require either dose adjustments or a switch to insulin. The list of adverse reactions is extensive, and includes central nervous system problems, dizziness, mild drowsiness, heart burn, changes in taste, changes in appetite, mild nausea, vomiting, stomach pain, fullness or discomfort in the stomach, constipation, frequent urination or increased urine output, and skin reactions, among others. Hematological reactions, although rare, may be severe and include aplastic anemia and hemolytic anemia. The administration of oral hypoglycemic drugs has been associated with increased cardiovascular mortality as compared with treatment with diet alone or diet plus insulin, but it is recommended that because their presence in breast milk might cause hypoglycemia in the newborn, breastfeeding be avoided while taking sulfonylureas.

Alpha-Glucosidase inhibitors are most common adverse effects are gastrointestinal problems, including flatulence, diarrhea, and abdominal pain. Alpha-glucosidase inhibitors may be excreted in small amounts in breast milk, and it is recommended that the drugs not be administered to nursing mothers.

Metformin causes gastrointestinal reactions in about a third of patients. A rare, but very serious, reaction to metformin is lactic acidosis, which is fatal in about 50% of cases. The symptoms of lactic acidosis are weakness, trouble breathing, abnormal heartbeats, unusual muscle pain, stomach discomfort, light-headedness and feeling cold. Patients at risk for lactic acidosis include those with reduced function of the kidneys or liver, congestive heart failure, severe acute illnesses, and dehydration.

Meglitinides, these drugs are generally well tolerated, with an adverse event profile similar to placebo. Thiazolidinedione, these drugs are generally well tolerated. However, they are structurally related to an earlier drug, troglitazone, which was associated with liver function problems. It is strongly recommended that all patients treated with pioglitazone or rosiglitazone have regular liver function monitoring.
12.2. Antioxidants treatments

Oxidative damage in the body is akin to rusting of metal, the browning of freshly cut apples, or fats going rancid. Certain substances known as antioxidants, however, can help prevent this kind of damage. Antioxidant protection as a defense against oxidative damage, the body normally maintains a variety of mechanisms to prevent such damage while allowing the use of oxygen for normal functions. Such “antioxidant protection” derives from sources both inside the body (endogenous) and outside the body (exogenous). Endogenous antioxidants include molecules and enzymes that neutralize free radicals and other reactive oxygen species, as well as metal-binding proteins that sequester iron and copper atoms.

Exogenous antioxidants obtained from the diet also play an important role in the body’s antioxidant defense. These include vitamin C, vitamin E, carotenoids such as β-carotene and lycopene, and other phytonutrients, or substances found in fruits, vegetables, and other plant foods that provide health benefits. Vitamin C and vitamin E are especially effective antioxidants because they quench a variety of reactive oxygen species and are quickly regenerated back to their active form after they neutralize free radicals. Vitamin C, which is abundant in fruits and vegetables, vitamin E, found in nuts, seeds, vegetable oils, and wheat germ, among other foods. Carotenoids, which are colored nutrients found in fruits and vegetables, provide their own unique antioxidant protection apart from vitamins C and E. The biological effects of free radicals are normally controlled in vivo by a wide range of antioxidants such as vitamin A, C and E, glutathione and antioxidant enzymes. Vitamin E, the main antioxidant in human beings, scavenges peroxyl-radicals, produced during lipid peroxidation (313). Reduced glutathione and vitamin C regenerate vitamin E (313). Vitamin A and C have also the ability to react directly with reactive oxygen species. Among antioxidant enzymes, superoxide dismutase (SOD) catalyzes dismutation of the superoxide anion (O2−) into H2O2, and glutathione peroxidase (GSH-Px) and catalase (CAT) both detoxify H2O2 and convert lipid hydroperoxides to nontoxic alcohols (313, 314) (Fig. 8).

Lipoic acid is an antioxidant capable of thiol-disulfide exchange. It is able to scavenge ROS and reduce metabolites such as glutathione to maintain a healthy
cellular redox state (315). It distributes to the mitochondria and serves as a critical cofactor for the mitochondrial enzyme complexes. Lipoic acid supplementation completely prevents diabetes-induced increase in nitrotyrosine and activation of NF-κB while decreasing the levels of VEGF and oxidatively modified proteins in the rat retina (316, 317). This antioxidant also inhibits diabetes-induced decreases in retinal mitochondrial and cytosolic ratios of NAD+ to NADH (318). Long-term administration of lipoic acid prevents the development of diabetic retinopathy in rats (316). Green tea, rich in polyphenols with great antioxidant potency, inhibits lipid peroxidation, and scavenges hydroxyl and superoxide radicals (319). Green tea supplementation in diabetic rats is reported to improve the levels of SOD and GSH, reduce the serum glucose levels (320). Trolox is a water soluble analog of vitamin E with potent antioxidant properties. Trolox is shown to partially prevent the loss of pericytes in diabetic rats via reducing membrane lipid peroxidation (321). Nicanartine, an antioxidant with cholesterol lowering properties, can partially inhibit pericyte loss in diabetic rats (322).

Dietary supplementation with multiple antioxidants comprising of vitamins C and E in diabetic rats prevents inhibition in retinal glutathione reductase, glutathione peroxidase, and SOD activities (323). Superoxide production in the retina is repressed by the same combination of vitamins in diabetic rats (320). Partial reductions in the development of retinal cellular capillaries and pericyte ghosts are seen in diabetic rats given the combination of vitamins C and E (320, 324). The benefits pertaining to retinal cells survival are more profound in diabetic rats consuming multiple antioxidants containing more components including ascorbic acid, \( \alpha \)-tocopherol acetate, trolox, N-acetyl cysteine, \( \beta \)-carotene, and selenium. Besides decreasing microvascular lesions, the multiple antioxidants abrogate the diabetes-induced increases in retinal PKC and NO (324). The same components of multiple antioxidants other than decreasing retinal PKC activity also reduce lipid peroxide, and prevent the decrease in SOD, glutathione reductase, and catalase activities in retina (325).

Zinc, a trace element with antioxidant properties, is shown to prevent diabetes-induced glutathione loss in the retina (326). Further, another trace element, selenium is reported to down-regulate VEGF production in the retina in diabetes (327). The various mechanisms by which Selenium may modify thyroid function, fertility and glucose homeostasis. The biological roles ascribed to selenium
include the prevention of cancer (328) cardiovascular disease (329, 330) and viral mutation (331). In addition the trace element is essential for optimal endocrine and immune function and moderating the inflammatory response (332, 333). Selenium has insulin-mimetic properties in vitro and in vivo. Insulin-stimulated glucose metabolism is impaired in adipocytes isolated from Selenium-deficient rats (334). An insulin-like effect of Selenium in cultured rat adipocytes includes stimulating glucose transport (335). When administered streptozotocin into diabetic rats, Selenium restores glycemic control and modifies the activity of a range of enzymes involved in hepatic glycolysis and gluconeogenesis. These changes are not linked to changes in insulin levels (336, 337, 338). In animal models, Selenium also prevents diabetes induced cardiomyopathy (338, 339, 340), renal and platelet function (341, 342). Selenium may exert these insulin-like effects on glucose metabolism by stimulating the tyrosine kinases involved in the distal signalling of the insulin signalling cascade (343, 344, 345).

Since diabetic cardiomyopathy is a multifactorial disease mainly due to oxidative and nitrosative stress, where more than one types of ROS and RNS are involve. Till date not a single antioxidant are effective against the generation or scavenging of ROS and RNS, so my aim and objective was to study the effect of multiple oxidants treatments in diabetic cardiomyopathy.