Chapter 1

Introduction

Diabetes mellitus is the disorder of glucose metabolism characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion and/or in insulin action (1). Therefore, although diabetes is an endocrine disease in origin its major manifestations are those of a metabolic disease. The word "diabetes" denotes the excessive urination in the disease. Areteaus, a Cappadocian physician of the 2nd century A. D. said "The epithet diabetes has been assigned to the disorder, being something like passing of water by a siphon". He perceptively characterized diabetes as "being a melting-down of the flesh and limbs into urine" (2). The word "mellitus" derives from Latin, meaning "sweetened with honey" and refers to the presence of sugar in urine of patients having the disease.

Amongst metabolic diseases, diabetes is considered to be one of the most prevalent throughout the world. The following Tables 1 and 2 show the population distribution and age distribution of diabetes mellitus in USA. As can be noted, about 6 % of the total population is diabetic in USA and percentage incidence of diabetes increases with age.

Table 1. Population distribution (%) of diabetes mellitus in the USA.

% Distribution	Category
15.7 million (5.9 % of population)	Diabetic
10.3 million	Diagnosed
5.4 million	Undiagnosed

Table 2. Age distribution (%) of diabetes mellitus in the USA.

Age group (in years)	% Distribution of diabetes
Under 20	0.16 %
20 and above	8.2 %
65 and above	18.4 %

Prevalence of diabetes: The global scenario

Stress on health education and life style changes may have in the years to come some favorable effect on the developed countries, for the projected raise in prevalence is relatively less - 27% from 6 to 7.6% in these countries compared to the developing World - 48% from 3.3 to 4.9% (Table 3). The World Health Organization (WHO) estimated that there were 135 million diabetic individuals in the year 1995 and it has projected that this number would increase to 300 million by the year 2025 (Table 3). It also declared that diabetes had reached epidemic proportions and that most of the increase will be contributed by developing countries, particularly India (3).

Prevalence of diabetes: The Indian scenario

Today India leads the world with its largest number of diabetic subjects in any given country. It has been estimated that in year 1995, 19.4 million individuals were found to be affected by diabetes and this number is expected to increase to 57.2 million by the year 2005 (one-sixth of the world total) (Table 3) (3).

Table 3. Top Ten Countries for Estimated Number of Adults with Diabetes in Millions

	Country	**1995**	**Country**	**2025**
1	India	19.4	India	57.2
2	China	16.0	China	37.6
3	USA	13.9	USA	21.9
4	Russian Federation	8.9	Pakistan	14.5
5	Japan	6.3	Indonesia	12.4
6	Brazil	4.9	Russian Federation	12.2
7	Indonesia	4.5	Mexico	11.7
8	Pakistan	4.3	Brazil	11.6
9	Mexico	3.8	Egypt	
10	Ukraine	3.6	Japan	8.5
All other country		49.7		103.6
Total		135.3		300.0

The crude prevalence rate of diabetes in **India** in urban areas is about 9% and the prevalence in rural areas has also increased to around 3 % of the total population. The 2003 WHO report has shown that there is a marked increase in the number of people affected with diabetes and this trend is scheduled to grow in geometric proportions in the next couple of decades. (Table 4) (3).

Table 4. Prevalence of diabetes in India.

Year	Author	Place	Prevalence (%)	
			Urban	Rural
1977	Ahuja	New Delhi	2.1	
1988	Ramachandran et al.	Kudremukh	5.0	
1992	Ramachandran et al.	Chennai	8.2	2.4
1997	Ramachandran et al.	Chennai	11.6	
2000	Kutty et al.	Thiruvananthapuram	12.4	
2001	Misra et al.	New Delhi	11.2	
2001	Ramachandran et al.	Six urban cities (DESI study)	12.1	

In the USA the (%) incidence of diabetes mellitus in all races is higher in females than in males (Table 5) (4).

Table 5. Distribution for causes of Type I diabetes mellitus (%) in the USA

Race/Gender	**Female**	**Male**
Alaskan, American Indian	0.2	0.1
Asian, Pacific Islander	0.4	0.3
Black	19.6	12.0
Hispanic	2.1	1.6
White	30.2	28.1
Other or unknown	2.8	2.6

Classification

The major classification of diabetes mellitus into clinical classis and statistical risk classes is shown in Table 6 and the characteristics of each subclass briefly described bellow.

Clinical classes

People of clinical classes show abnormality of glucose tolerance. Patients of clinical classes are further classified in three subclasses.

(i) Diabetes mellitus, (ii) Impaired glucose tolerance (IGT) and (iii) Gestational diabetes mellitus (GDM).

(i) Diabetes mellitus

Diabetes mellitus further divided in following classes.

Type 1 diabetes, which is insulin-dependant diabetes mellitus (IDDM) results from cellular-mediated autoimmune destruction of pancreatic islet beta-cells causing the loss of insulin production (5). It ranks as the most common chronic childhood disease in developed nations (6), but occurs at all ages (7) and the clinical presentation can vary with age (8, 9).

Type 1 diabetes in an adult may masquerade as type 2 diabetes at presentation with a slow deterioration in metabolic control, and subsequent progression to insulin dependency. This form is called latent autoimmune diabetes mellitus in adults (LADA) (10). LADA falls within type 1 autoimmune diabetes, but in a slowly progressive form, in the new WHO classification (10).

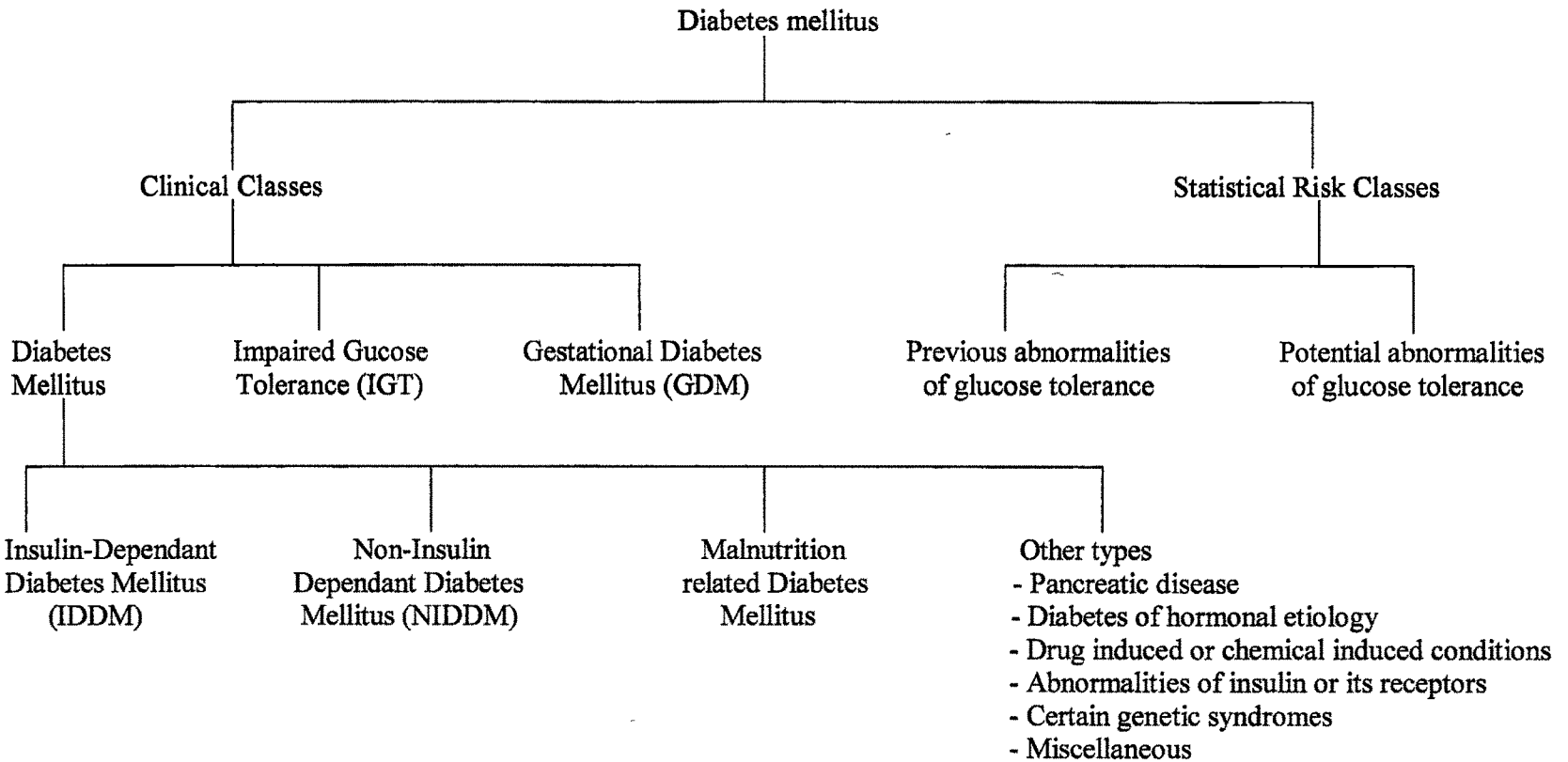
The predominant cause of hyperglycemia in type 1 diabetes is the autoimmune destruction of the beta cells, which leads to absolute dependence on insulin treatment and a high rate of complications typically occurring at relatively young ages. Type 1 diabetes, therefore, places a particularly heavy burden on the individual, the family and the health services.

Type 2 diabetes mellitus, also known as non-insulin-dependant diabetes mellitus (NIDDM) is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest (11, 12). The specific reasons for the development of these abnormalities are not yet known.

Usually type 2 diabetes diagnosed after the age of 40 years although, the age of onset is often a decade earlier in populations with high diabetes prevalence (13). People with type 2 diabetes may not show any symptoms for many years and the diagnosis is often made from associated complications or incidentally through an abnormal blood or urine glucose test.

Type 2 diabetes is often, but not always, associated with obesity, which itself can cause insulin resistance and lead to elevated blood sugar levels. It is strongly familial, but major susceptibility genes have not yet been identified. In contrast to type 1 diabetes, persons with type 2 diabetes are not dependent on exogenous insulin and are not ketosis-prone, but may require insulin for control of hyperglycemia if this is not achieved with diet alone or with oral hypoglycemic agents.

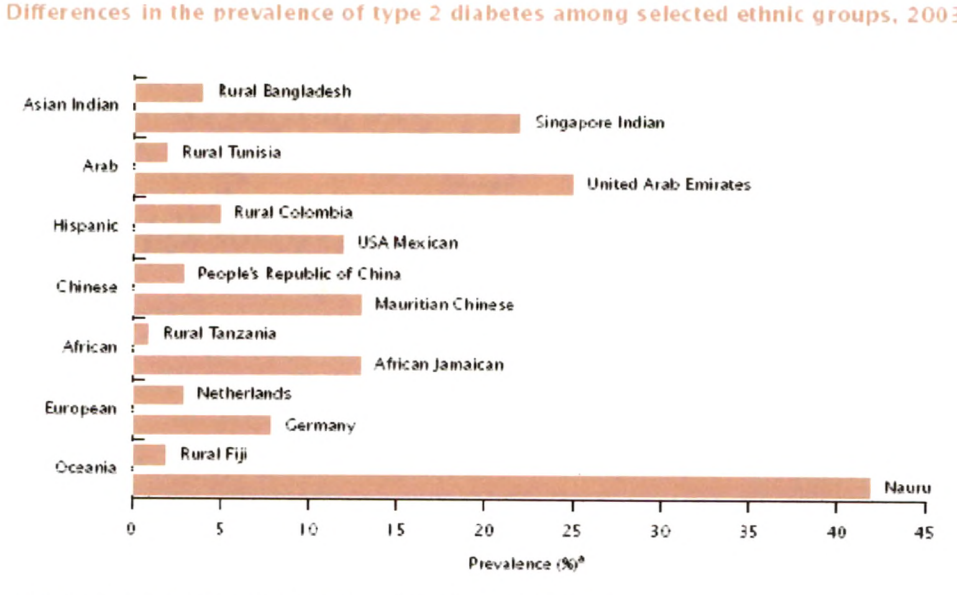
Table 6. Classification of diabetes mellitus (14).



Type 2 diabetes constitutes about 85 to 95% of all diabetes in developed countries, and accounts for an even higher percentage in developing countries. It is now a common and serious global health problem, which, for most countries, has evolved in association with rapid cultural and social changes, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyles and behavioral patterns (15)

Fig 1 below highlights the large range of prevalence of type 2 diabetes even within the same or similar ethnic groups, when living under different conditions. Clearly, many of the differences between these rates reflect underlying behavioral, environmental and social risk factors, such as diet, level of obesity and physical activity.

Figure 1.



a. Rates are age-standardized to Segi's World Population for ages 30 to 64 years

Adapted from King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. Diabetes Care 1993; 16:157-177.

Source: *Diabetes Atlas* second edition, ©International Diabetes Federation, 2003

Malnutrition related diabetes mellitus: Forms of diabetes due to malnutrition.

Other types involve **Pancreatic disease:** Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. Cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion (16, 17). **Diabetes of hormonal etiology:** Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved (16, 17). **Drug induced or chemical induced conditions:** Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance (16, 17). **Abnormalities of insulin or its receptors:** There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. (16, 17). **Certain syndromes:** Leprechaunism and the Rabson- → Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance (16, 17) and **Miscellaneous.**

(ii) Impaired glucose tolerance:

The Expert Committee (3, 18) recognized an intermediate group of subjects whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. This group is defined as having fasting plasma glucose (FPG) levels ≥100 mg/dl (5.6 mmol/l) but <126 mg/dl (7.0 mmol/l) or 2-h values in the oral glucose tolerance test (OGTT) of ≥140 mg/dl (7.8 mmol/l) but < 200 mg/dl (11.1 mmol/l). Thus, the categories of FPG values are as follows:

- FPG <100 mg/dl (5.6 mmol/l) = normal fasting glucose;
- FPG 100–125 mg/dl (5.6–6.9 mmol/l) = IFG (impaired fasting glucose);
- FPG ≥126 mg/dl (7.0 mmol/l) = provisional diagnosis of diabetes

Patients with IFG and/or IGT are now referred to as having "pre-diabetes" indicating the relatively high risk for development of diabetes in these patients. In the absence of pregnancy, IFG and IGT are not clinical entities in their own right but rather risk factors for future diabetes as well as cardiovascular disease.

(iii) Gestational diabetes

The most widely accepted definition of gestational diabetes mellitus (GDM) is "carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy" (19, 20). This definition applies regardless of whether insulin is used for treatment or the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have occurred before the pregnancy.

It is widely believed that differences in reported prevalence of GDM parallel the differences that have been found in the frequency of type 2 diabetes among different populations. Nonetheless GDM is increasing in prevalence in concert with the worldwide rise in type 2 diabetes.

Statistical risk classes

These are further sub-classified in to two

(i) Previous abnormality of glucose tolerance includes people who had abnormality of glucose tolerance in the past.

(ii) Potential abnormality of glucose tolerance: Diabetic relatives and obese subjects are at increased risk for development of diabetes mellitus, and therefore are classed as potential abnormality of glucose tolerance (POT-AGT) (21).

Possible causes for type 1 diabetes:

These include (i) Some type of destructive immune response i.e. having antibodies against our own beta cells, (ii) Viral infection and (iii) Environmental factors.

Possible causes for type 2 diabetes:

These include (i) Associated with obesity and (ii) Insulin is usually produced but cell may not respond to hormone due to the presence of defective insulin receptor (22).

The characteristic symptoms in diabetes are:

- Hyperglycemia
- Glucosuria
- Polyphagia
- Polydipsia
- Polyuria

Diabetics also have a high risk of developing long term diabetes complications (23), including:

(I) Microvascular diseases

- Nephropathy
- Angiopathy (Macro- and Micro-angiopathy)
- Retinopathy
- Neuropathy

(II) Macrovascular diseases

- Cardiovascular
- Peripheral Vascular Disease
- Ceribrovascular

The developments of diabetic complications do not depend entirely on duration of diabetes and control. Predisposing and aggregative factors, either constitutional or environmental, seem to play a role (24, 25).

Details of diabetic complications are briefly described below:

Diabetic Nephropathy

There are four general theories or processes that have been advanced in recent years to explain the pathology of diabetic nephropathy. These are as follows:

(a) Glomerular homodynamic changes with elevations of flows and pressures occur early in the course of diabetes and these have been suggested to be directly responsible for the development of glomercular sclerosis and proteinuria. But the role of these local factors is being debated (26).

(b) Structure-function alterations resulting from non-enzymatic glycation of basement membrane macromolecules and other proteins (27-30).

(c) Increased flux through insulin – independent pathways for glucose utilization, particularly the polyol pathway (27, 28).

(d) Disturbed regulation of the synthesis or metabolism of specific basement membrane components, in particular collagen and proteoglycans (31).

Formation of excess amount of advanced glycation end products (AGEs) and increased flux through polyol pathway and their consequences as mechanisms are two major pathways contributing to the development of diabetes nephropathy.

Diabetic Retinopathy

The diabetic retina shows abnormalities of blood flow although the precise role of these abnormalities remains in doubt. Similarly a variety of abnormalities in rheology and coagulation can be demonstrated in patients with diabetic retinopathy. Blood viscosity

was significantly higher in diabetics than in controls. No significant differences in viscosity of the whole blood were found when various types of retinopathy were compared according to the severity of retinal damage. Plasma viscosity was significantly higher than controls only in diabetic patients with retinopathy. Serum viscosity was significantly increased compared with controls only in diabetic patients affected by proliferative retinopathy (32). Platelet activity is increased at the site of vessel injury (33).

Diabetic Cataract:

Because of their avascular nature and extremely low regenerative potential, mammalian lenses are very susceptible to damage associated with metabolic disorder such as diabetes. Increase in the concentration of polyols, lens fiber swelling, loss of intracellular metabolites, decrease in glutathione concentration, and increase in protein glycation are the general features of the lenses in diabetes (34-36). A frequent pathological end point of this multifactorial degenerative process is the production of cataracts (37).

Diabetic Neuropathy:

Neuropathy is one of the most debilitating complications of both type 1 and type 2 diabetes. Multiple factors are playing a major role in the pathogenesis of diabetic neuropathy, which include reduced energy utilization (38), increased sorbitol concentration and decreased nerve free myo-inositol concentrations (38, 39), increased intra-axonal sodium level (40), and a reduced rate of incorporation of lipid and amino acids in myelin (41). Other than these, alterations in the endoneural metabolism,

defective neuropathic factor, reduce nerve blood supply and immune mechanisms. These are because of excessive stress, the polyol pathway, excess formation of advanced glycation end products (AGEs), protein kinase C and impaired essential fatty acid metabolism. In diabetic neuropathy there is variable involvement of large myelinated fibers and small, thinly myelinated fibers. (42). In diabetes there is a deficiency of nerve growth factor (NGF), as well as the neuropathies substance P (SP) and calcitonin gene-related peptide (CGRP) which contribute to the clinical symptoms resulting from small fibers dysfunction (42). For large fiber NT3 appears to be important and IGFs for autonomic neuropathy (42). Diabetes mellitus is associated with cognitive deficits and an increased risk of dementia, particularly in the elderly. These defects are paralleled by neurophysiological and structural changes in the brain (43).

In animal models of diabetes, impairments of special learning occur in association with distinct changes in hippocampal synaptic plasticity have been reported (43). At the molecular level these impairments might involve changes in glutamate receptor subtypes, in second messenger systems and in protein kinases (43, 44). Axonal transport rates are also reduced in experimental diabetic neuropathy (45). While the link between these abnormalities and development of diabetic neuropathy is still not clear, all are energy-dependent processes that might be impaired if the nerve microenvironment were hypoxic (46). In diabetic animals, hyperglycemia and resultant increased sorbitol pathway activity in peripheral nerve is associated with decreased oxygen uptake in this tissue (47). Such impairment of tissue oxygen delivery could conceivably contribute to the development of neurological dysfunction (48).

Cardiovascular Disease and Diabetes

Cardiovascular disease (CVD) is a major cause of mortality in individuals with diabetes. Many factors including hypertension contribute to the high prevalence of CVD in diabetic population (49). The risk factors for CVD in diabetic patients include atherosclerosis, dyslipidemia, microalbuminuria, endothelial dysfunction, platelet hyperaggregability, coagulation abnormalities and diabetic cardiomyopathy (49).

Patients with diabetes mellitus have a grater than 3-folds increased risk of coronary ischemic events and congestive heart failure. Diabetes is associated with profound changes in cardiac metabolism, characterized by diminished glucose utilization, diminished rates of lactate oxidation and increased use of fatty acids (50). Fatty acid oxidation is an important source of energy in heart. In diabetes fatty acid oxidation dramatically increases in heart and can account for almost 100 % of the heart's energy production (51). Diabetes is associated with an increased or poorly regulated rate of amino acid catabolism in the heart. The incidence of coronary artery disease correlates more closely with duration of diabetes than with the severity of diabetes.

Accelerated large vessel disease (macro-angeopathy) in diabetes may be due in part to abnormalities in plasma lipids, and perhaps also to changes in the composition and metabolism of the arterial wall. In macro-angeopathy, early histological studies have revealed an accumulation of hyaline substances along with deposition of cholesterol crystals, calcium and increased amounts of glycoprotein (52). Such a deposition, which may result in hardened arteries and a narrowed arterial lumen, superimposed with

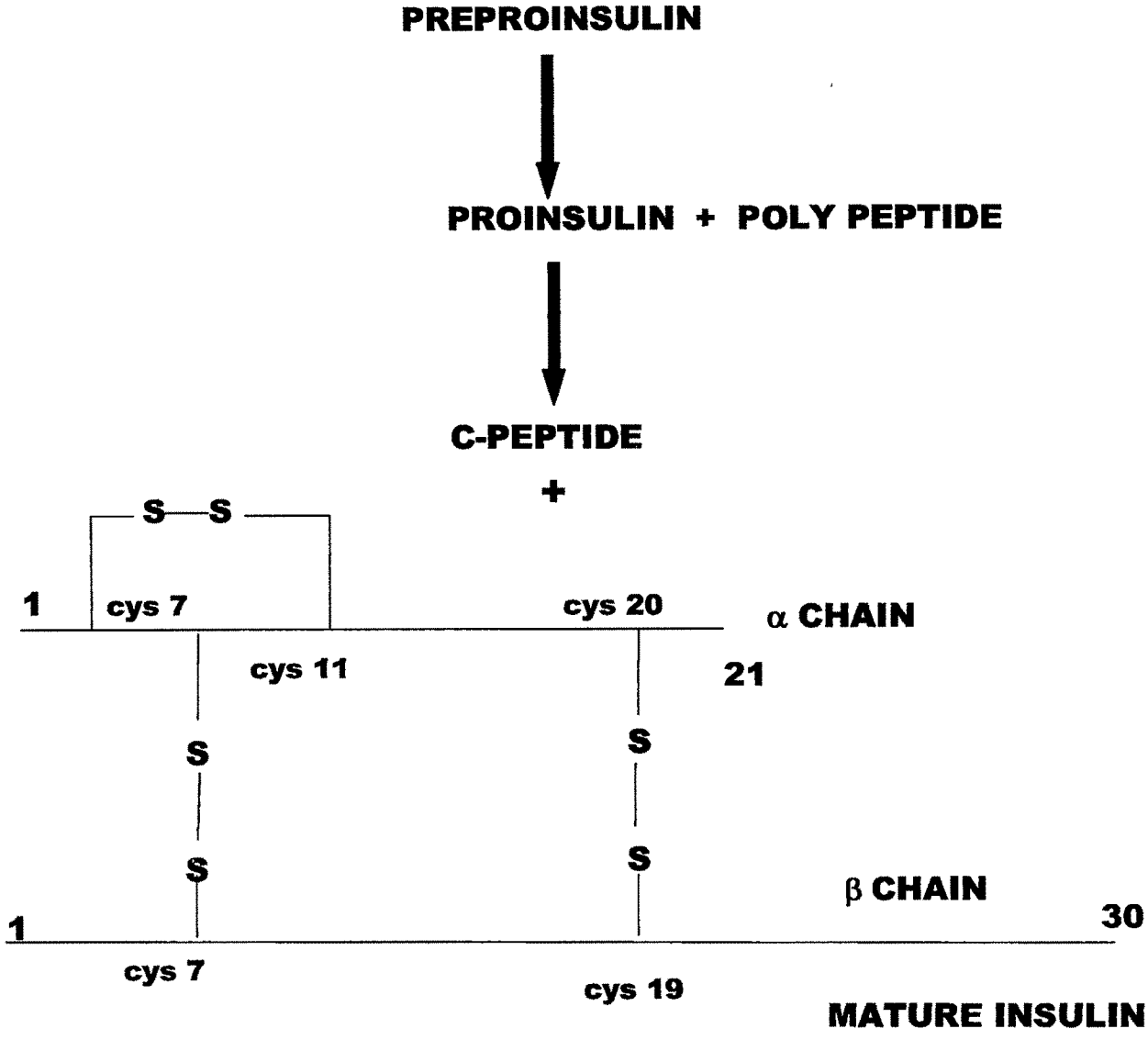
atherosclerosis could easily account for the angina and myocardial infarction associated with diabetes (53).

Not only is the frequency of acute myocardial infarction increased in diabetic patients (54), but also the treatment of the infarct is more complicated than in the non-diabetics. Insulin-dependant diabetes mellitus appears to increase the likelihood of the development of congestive heart failure (CHF) from all causes.

Insulin

The complex interlinking of different facets of metabolism requiring rapid adaptive changes in environment, particularly the changes from the fasted to the fed state, necessitates a prompt and clear signal. This signal is provided by insulin (55). Insulin is released from beta cells of islets of Langerhans present in the pancreas. It originates as pre-pro-insulin. In endoplasmic reticulum 23 amino acids get separated and form pro-insulin. Pro-insulin goes to Golgi and c-peptide gets separated from A and B chains. Mature insulin is a polypeptide of 39 amino acids arranged as A and B chains joined by one intra- and two inter-peptide disulfide bridges. The double chain structure results from its origin as pro-insulin, when a peptide length joins the end of the A to the B chain. The connecting peptide chain, c-peptide secreted in equimolar amounts with insulin, appears to be inert and in contrast to insulin is excreted virtually unchanged in urine (55). However, c-peptide has now been shown to have insulin-like action and corrects many of the maladies associated with diabetes (56-59); c-peptide dose not have blood sugar level

Figure 2 **MATURATION OF INSULIN**



lowering effect (56-59). Fig. 2 schematically represents the steps in the synthesis of mature insulin from its precursor, pre-pro-insulin.

The physiologic effects of insulin in mammalian system include stimulation of hexose, ion and amino acid uptake (60), modification of the activities of rate-limiting enzymes such as glycogen synthase, hormone sensitive lipases and pyruvate dehydrogenase by net dephosphorylation (61). Insulin regulates the gene expression for a small number of regulatory enzymes (62), redistribution of membrane proteins such as the glucose transporters and the insulin-like growth factor II (IGF-II) and transferrin receptors (63), and promotion of cell growth (64). Many of these effects are tissue- or cell-specific and involve only a discrete subset of proteins. The chronology varies. Transduction of the gene encoding phosphoenolpyruvate carboxikinase is inhibited within seconds of addition of insulin, whereas growth promotion requires hours of exposure (Table 7).

Many of the rapid actions of insulin, such as stimulation of hexose transport alteration and alteration of the enzyme activities do not depend on synthesis of new proteins or nucleic acids. Even this incomplete summary of the action of insulin, however invokes seryl and threonyl phosphorylation and dephosphorylation of cytosolic and mitochondrial proteins, membrane translocations with the likelihood of cytoskeletal proteins involvement, and nuclear action (65).

Table 7. Chronology of insulin action

Seconds
Binding to receptor
Activation of receptor protein tyrosine kinase
Receptor autophosphorylation
Seconds to minutes
Changes in gene transcription
Stimulation of hexose and ion transport
Ligand-mediated receptor internalization
Alterations in intracellular enzyme activities
Seryl and threonyl phosphorylation of the receptor
Hours
Synthesis of protein, lipid and nucleic acids
Maximal down-regulation of the receptor
Cell growth

Molecular mechanism of insulin action

The first essential and common step in insulin action is interaction with the insulin receptors. Insulin delivers its signal through binding to its high-affinity cell surface receptors. This protein is a tetrameric complex consisting of two α and two β subunits (65, 66). All the subunits link with each other by inter- and intra-peptide disulfide bridges (65). α subunit is present on the outer surface of the cell, while the β subunit is a trans-membrane subunit (65). The intracellular domains of β subunit contain intrinsic protein tyrosine kinase activity and are involved in the initiation of insulin-dependant transmembrane signaling events. The early signal transduction events involve the autophosphorylation of its receptor on tyrosine residues and of the insulin receptor substrates 1, 2 and 3 (IRS – 1, -2 and -3), leading to the generation of docking site on both insulin receptor and the IRS proteins for SH2 and SH3 domain – containing proteins. These proteins which include both enzymes and adapters initiate a multitude of downstream signals that regulate the phosphorylation state of a wide verity of proteins and some phospholipids (66).

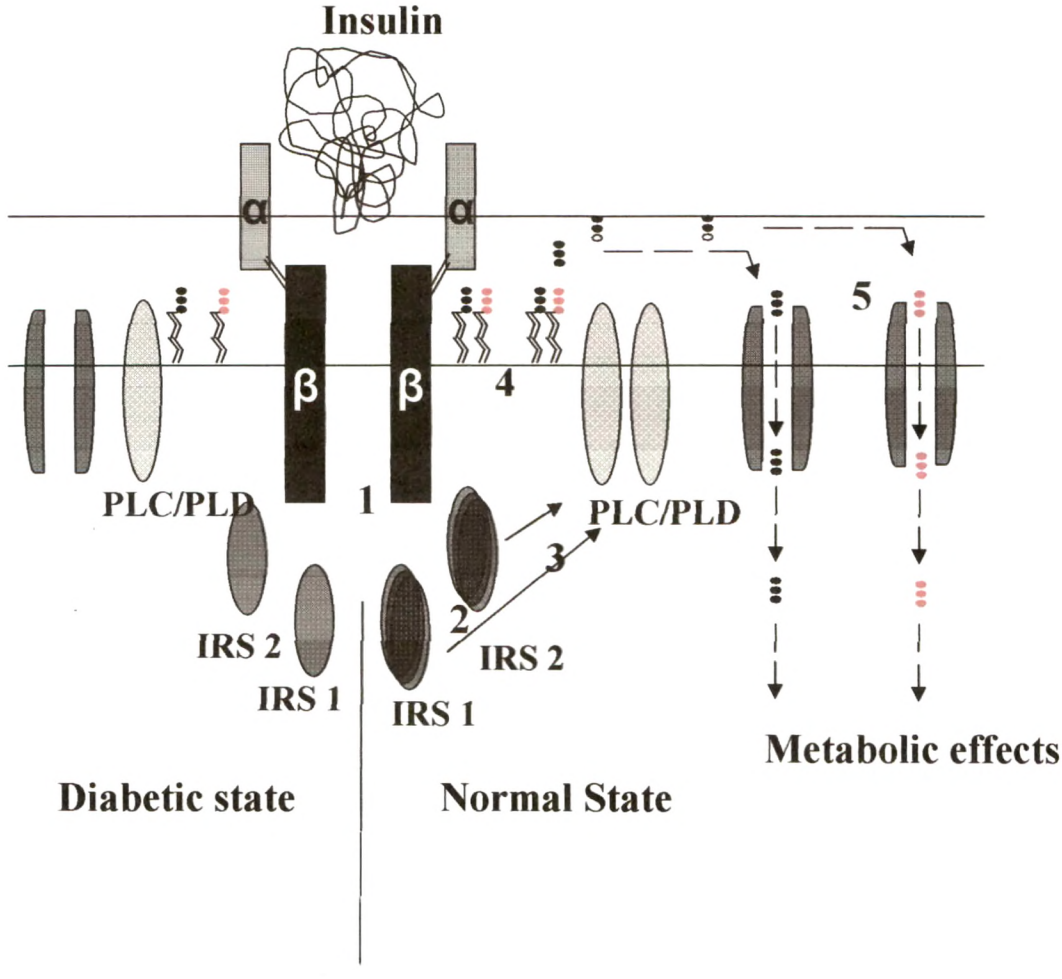
A possible route through which insulin could stimulate inositol phosphoglycan (IPG) type-A generation in normal insulin responsive cell is depicted in Fig. 3 (66).

Points within the proposed route at which a blockade would result in the formation of insulin resistant cells are indicated.

1. Insulin receptor protein tyrosine kinase (point-mutated, kinase domain-deleted, serine-threonine hyper-phosphorylation).
2. IRS protein (expression levels, phosphorylation state)
3. Phospholipase C (PLC) or D (PLD) (expression level coupling)
4. GPI in the plasma membrane (quantity, localization)
5. IPG type-A transporter receptor, IPG type-A signal 'transducing protein' (expression level, functionality).

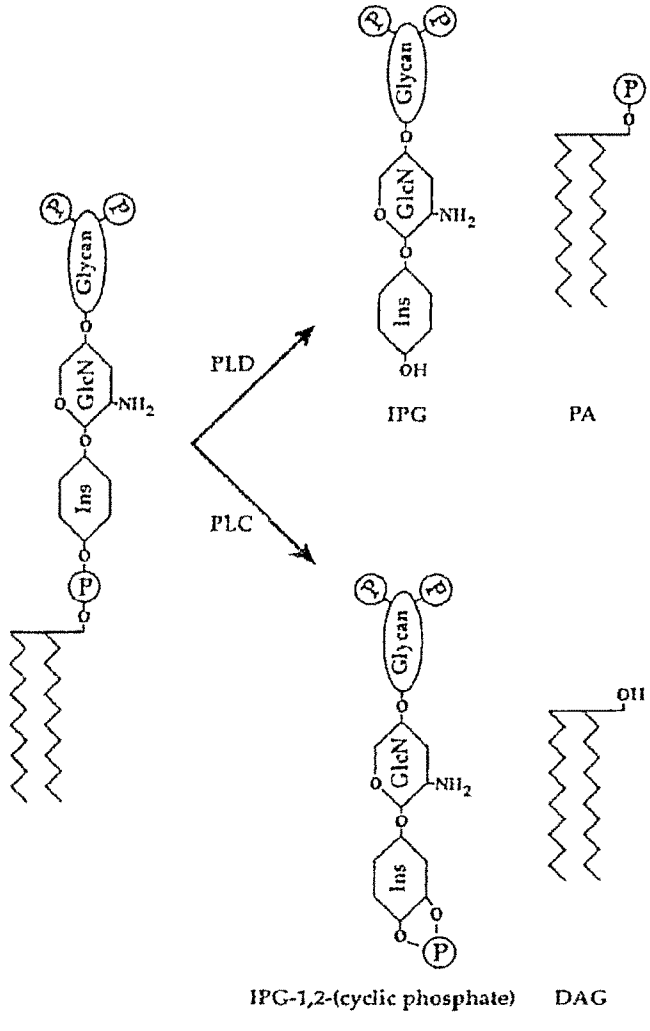
The **black** symbols represent IPG derived from GPI-PLD-mediated GPI hydrolysis. The **red** symbols represent IPG derived from GPI-PLC-mediated GPI hydrolysis.

Figure 3 Schematically depicts the mechanism of insulin action



The Fig. 4 is a schematic representation of the structure and hydrolysis of a GPI phospholipid by phospholipase D (PLD) and C (PLC) generating IPG type A and PA, and IPG-1, 2-(cyclic phosphate) type A and DAG.

Ins, myo-Inositol; GlcN, Glucosamine; PA, Phosphatidic acid; DAG, Diacyl glycerol (66).



IPG type A itself can mimic a large number of metabolic action of insulin which are listed in Table 8 (66).

Table 8. Insulin mimetic effects of IPG type A (A), IPG type P (P), and their analogues from different sources

Whole Cells		Cell Extracts	
Biological Activity	**Effect**	**Enzymatic Activity or Phosphorylation**	**Effect**
Lipolysis (A, A*, A°)	Inhibition	cAMP phosphodiesterase (A)	Stimulation
Lipogenesis (A, A*, A°)	Stimulation	Pyruvate dehydrogenase (A)	Stimulation
GPAT (A*, A°)	Stimulation	PDH phosphatase (P)	Stimulation
Phospholipid methyltransferase (A)	Inhibition	Glucose-6-phosphatase (A)	Inhibition
Steroidogenesis (A, P*)	Stimulation	Fructose-1,6-biphosphatase (A)	Inhibition
Glucose transport (A, A*, A°)	Stimulation	Adenylate cyclase (A)	Inhibition
GLUT4 translocation (A*, A°)	Stimulation	cAMP-kinase (A)	Inhibition
Acetyl-CoA carboxylase (A)	Stimulation	Casein kinase II (A)	Biphasic
Glycogen phosphorylase a (A)	Inhibition	Glycerol-3P acyltransferase (P)	Stimulation
Pyruvate kinase (A)	Stimulation	ATP citrate lyase (A)	Stimulation
Glucose oxidation (A)	Stimulation	Galactolipid sulfotransferase (A)	Inhibition
Glucose production (A)	Inhibition	Protein phosphatase 2C (P)	Stimulation
Lactate accumulation (A)	Stimulation		
Glycogen synthesis (A, P, A*, A°)	Stimulation		
Glycogen synthase kinase-3 (A*, A°)	Inhibition		
Tyrosine aminotransferase (A)	No effect		
Protein phosphorylation (A, A*, A°)	Stimulation Inhibition		
cAMP levels (A)	Inhibition		
PI 3-kinase (A*, A°)	Stimulation		
Myelin basic protein kinase (A°)	Stimulation		
Mitogen activated kinase (A°)	Stimulation		
Protein kinase B phosphorylation (A°)	Stimulation		
Fructose-2,6-P2 levels (A)	Stimulation		
Ion channels (A)	Modulation		
Ca^{2+}-Mg^{2+} ATPase (A)	Stimulation		
Ca^{2+} entry (A)	Inhibition		
Amino acid transport (A)	Stimulation		
Protein synthesis (A, A°)	Stimulation		
Specific mRNA levels (A)	Stimulation Inhibition		
DNA and RNA synthesis (A)	Stimulation		
Cell proliferation (A, P)	Stimulation		
Insulin secretion (A)	Inhibition		
Cell differentiation (neurogenesis) (P)	Stimulation		

Adapted and updated from Jones and Varela-Nieto (17) A*, A phosphoinositolglycan-peptide (PIG-P) (57,58,61); A°, various chemically synthesized PIG-P analogues (38,39), GPAT glycerol-3-phosphate acyltransferase P*, an analog of IPG type P (INS-2) consisting of α-pinitol and galactosamine (36) PDH pyruvate dehydrogenase

Forgoing review highlights that insulin-status significantly alters various tissues/organs in the body. Hyperglycemia and other altered metabolic processes in diabetes result in nephropathy, cardiomyopathy, retinopathy, cataract, neuropathy etc. Diabetic state also alters membrane structure-function relationship in the cell. In the present studies effects of alloxan-diabetes and subsequent insulin treatment have been examined at the subcellular levels i.e. mitochondria and microsomes. Hence a brief account of mitochondria, mitochondrial electron transport chain, mitochondrial membrane enzymes namely, FoF_1 ATPase and cytochrome oxidase, and on microsomes and microsomal enzymes Na^+, K^+-ATPase and glucose-6-phosphatase (G6Pase) is given below. A brief account of free radicals and reactive oxygen species (ROS) is also appended.

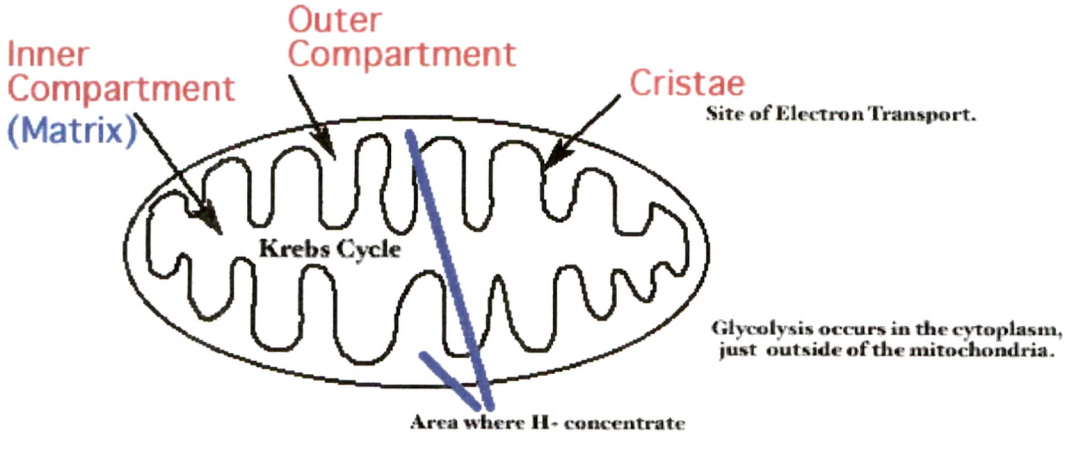
Mitochondria

The mitochondrion lies at the heart of cell life and cell death. A mitochondrion is typically long and slender, but it can appear bean-shaped or oval-shaped under the electron microscope. Ranging in size from 0.5 micrometer to 1 micrometer in length, a mitochondrion has a double membrane that forms a sac within a sac. The smooth outer membrane holds numerous transport proteins, which shuttle materials in and out of the mitochondrion. The region between the outer and inner membranes, which is filled with liquid, is known as the outer compartment. The inner membrane has numerous folds called cristae. Cristae are the sites of ATP synthesis, and their folded structure greatly increases the surface area where ATP synthesis occurs. Transport proteins, molecules called electron transport chains, and enzymes that synthesize ATP are among the molecules embedded in the cristae. The cristae enclose a liquid-filled region known as the

inner compartment, or matrix, which contains a large number of enzymes that are used in the process of aerobic respiration. That we must breathe oxygen to stay alive is simply the consequence of the demand of our mitochondria for oxygen. About 98% of inhaled oxygen is consumed by mitochondria, and without mitochondria, we would have no need of the oxygen transfer machinery of the lungs, red cells, hemoglobin, or even the circulatory system that delivers oxygen to the tissues (67). Similarly, the organization of food intake, digestion, and processing is designed primarily to supply substrates destined for mitochondrial oxidation. Impaired mitochondrial function will lead to disease, ranging from subtle alterations in cell function to cell death and from minor to major disability, or to death (67). Quite apart from the provision of ATP, mitochondria play important roles in aspects of normal physiology (67). These include the transduction pathway that underlies the secretion of insulin in response to glucose by β cells and (possibly but controversially) in the sensing of oxygen tension necessary for oxygen sensing in the carotid body and the pulmonary vasculature (67). Mitochondria also house key enzyme systems quite distinct from those required for intermediary metabolism--the rate-limiting enzymes in steroid biosynthesis, the synthesis of haem, and even the carbonic anhydrase required for acid secretion in the stomach (67). By accumulating calcium when cytosolic calcium levels are high, mitochondria play subtle roles in coordinating the complexities of intracellular calcium signaling pathways. At least in some cell types in which their contribution may be extremely important in the finer aspects of cell regulation (67). The physiological "uncoupling" of mitochondria plays a central role as a heat-generating mechanism in non-shivering thermogenesis in young mammals (67). It has also been suggested that the production of free radical species by

mitochondria may play a key role as a signaling mechanism, for example, in the regulation of ion-channel activities and also in initiating cyto-protective mechanisms in stressed cells (67). Mitochondrial damage in pancreatic β-cells causes diabetes. Mitochondrial dysfunction in the heart may give rise to cardiomyopathy (68) Indeed, the production of free radicals by mitochondria has been considered by many to play a central role in the degradation of cellular function that appears to underlie the process of aging, whereas some of the genes identified in the control of longevity appear to target mitochondria or at least to alter antioxidant defenses of the cell (69, 70). Accumulations of mitochondrial defects have been implicated as a mechanism of aging and age-related diseases (69).

Fig. 5 Structure of mitochondrion



Mitochondrial DNA (mt DNA)

The human mitochondrion contains 5-10 identical, circular molecules of DNA (71). Each consists of 16,569 base pairs which carries the information for 37 genes. These genes encode 2 different molecules of ribosomal RNA (rRNA), 22 different molecules of transfer RNA (tRNA) (at least one for each amino acid) and 13 polypeptides. The rRNA and tRNA molecules are used in the machinery which synthesizes the 13 polypeptides. These polypeptides are subunits of the protein complexes in the inner mitochondrial membrane, including subunits of NADH dehydrogenase, cytochrome c oxidase and ATP synthase (72). However, each of these protein complexes requires subunits that are encoded by nuclear genes, synthesized in the cytosol, and imported from the cytosol into the mitochondrion (72).

A number of human diseases are caused by mutations in genes in our mitochondria. The defects are mostly in cytochrome b, 12S r RNA, ATP synthase, subunits of NADH dehydrogenase or several tRNA genes. Although many different organs may be affected, disorder of the brain and muscles are most common (73)

Diabetes and mitochondria

It is becoming increasingly evident that diabetes can affect the mitochondrial functions in various tissues (74-76). Mutations in the glucokinase gene are responsible for one form of maturity-onset diabetes of the young (MODY2) (77), and the other type, mitochondrial diabetes mellitus (MDM), is associated with mutations of the mitochondrial DNA (mtDNA) affecting subunits of respiratory chain complexes (78).

Mechanisms that affect insulin secretion in response to blood glucose increase. According to a generally accepted scheme, insulin secretion in response to postprandial glucose elevation depends on a sequence of metabolic events: 1) the uptake of glucose through the GLUT2 transporter, 2) phosphorylation of glucose by glucokinase, 3) production of NADH and pyruvate by glycolysis, and 4) stimulation of mitochondrial oxidative phosphorylation (OXPHOS). GLUT2 is the major, if not the only, glucose transporter in the off-cells (C14). The K.sub.m of GLUT2 (17 mmol/l) is significantly higher than that of the ubiquitous GLUT1 or the fat and muscle tissue-specific glucose transporter GLUT4. GLUT2 is not rate-limiting, glucokinase (hexokinase IV), which has a high K.sub.m for glucose (>5 mmol/l) (79, 80). In isolated mouse islets, high glucose stimulates the oxidation rate, and inhibition of OXPHOS inhibits insulin secretion (81, 82). Blocking of a K^{+}.sup. channel by ATP is assumed to be responsible. The closure of the K^{+}.sup. channel results in cell membrane depolarization followed by subsequent influx of Ca^{2+}.sup. and stimulation of insulin exocytosis (83). The influence of cytosolic and mitochondrial calcium fluctuations on insulin secretion (84) has been confirmed in elegant studies by Rutter et al. (85). Using an aequorin-transfected INS-1 cell line challenged by ATP or depolarized by high K^{+}.sup, the authors demonstrated that the transient cytoplasmic Ca^{2+}.sup. increase is accompanied by elevation of mitochondrial Ca^{2+}.sup. concentration, more than one order of magnitude above the cytoplasniic Ca^{2+}.sup. levels. This should be sufficient to activate Ca^{2+}.sup.-sensitive intra-mitochondrial dehydrogenases and this increase in driving force of the respiratory chain should further promote ATP synthesis (85).

The mitochondrial tRNA.sup.Leu(UUR) gene is an etiologic hot spot for mtDNA mutations, as at least 10 disease-related mutations have been described so far in this gene (86). Four of them have been associated with diabetes and various other symptoms (87). In 1992, two independent publications appeared demonstrating an A/G exchange at np 3243 in the tRNA.sup.Leu(UUR) gene in large Dutch and British families with diabetes and deafness. These first reports were confirmed by several other groups (87-89). The tRNA.sup.Leu(UUR) mutation at np 3243 is found in ~0.5-1.5% of unselected diabetic patients, independently of whether they are classified as having type I or type II diabetes In diabetic patients with familial history, the percentage increases up to 10%. The prevalence seems not to be very different between various countries and races (87-89) Diabetes is rarely also found in association with the so-called Myoclonic epilepsey and ragged red fiber disease (MERRF) mutation at np 8344 in the tRNA.sup Lys gene (90, 91). Recently, a T14709C transition in the tRNA.sup.Glu gene was demonstrated independently by two groups in a syndrome with myopathy and diabetes (92, 93).

mtDNA length mutations and diabetes.

Mutations in mitochondrial DNA have been implicated in etiology of diabetes (89, 94) Distinct length abnormalities of the mtDNA were first described in 1988 in patients suffering from mitochondrial myopathies (muscle weakness, chronic progressive external ophthalmaplegia (CPEO) or the complete Kearns-Sayre syndrome (KSS) (95-100). Endocrine dysfunction, for example hypogonadism, hypothyroidism, hypoparathyroidism, and diabetes, was found in a high degree in KSS and CPEO (100-103). While partial deletions were the first mtDNA defects described in KSS, Poulton et

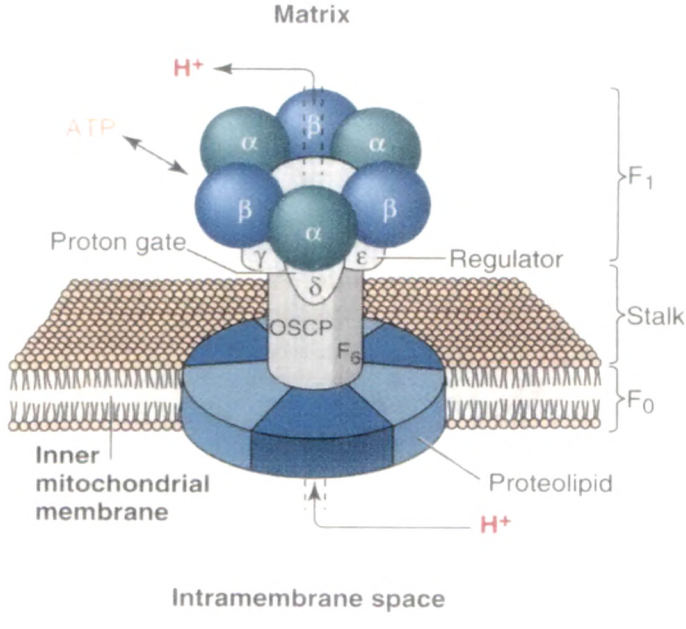
al. 1 year later reported partial direct tandem duplications (104). Further studies reinforced a characteristic association between partial duplication and diabetes (105-107). In 1992, Ballinger et al. described a large pedigree with a maternally inherited syndrome of diabetes and deafness carrying a 10.4-kb mtDNA, a mixture of interrelated and rearranged mtDNA, a mixture of interrelated and rearranged mtDNA molecules, namely duplications and deletion dimers, but few deletion monomers (108, 109). The proportion of each rearranged mtDNA molecule varies between different tissues, and there is growing evidence that the balance of mtDNA molecules, namely duplications and deletion dimers, but few deletion monomers (109). The proportion of each rearranged mtDNA molecule varies between different tissues, and there is growing evidence that the balance of mtDNA re-arrangements may be central to the pathogenesis of this form of mitochondrial diabetes mellitus (MDM) (105, 107).

FoF_1 ATPase

The FoF_1 ATPase (Complex V) which functions as ATP synthase *in situ* is an important enzyme system responsible for the conservation of energy released during substrate oxidation, in the form of ATP in mitochondria (110, 111). The FoF_1 ATPase contains the F_1 knob projecting into the matrix, which is connected by a stalk to the F_0 base embedded in the inner membrane (Fig. 6) (110, 111). It is generally considered that the mammalian mitochondrial ATP synthase complex is composed of 16 unlike subunits (111). These subunits are $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ and probably factor B in the catalytic F1 domain; OSCP, a, b, c, d, e, f, g, F6 and A6L in Fo and stator; and the ATPase inhibitor protein, IF1, which binds reversibly to F1 to inhibit ATP hydrolysis (110-112). Fo base contains highly variable number of a, b and c ($a_1b_2c_{10-14}$) subunit types depending on species and a proton

conducting pathway (111,112). Operating together, FoF_1 provides (Δp)-consuming ATP synthesis or (Δp)-generating ATP hydrolysis depending on the physiological (*in vivo*) or experimental (*in vitro*) conditions (110, 112, 113). FoF_1 ATPase is universally present as essential component of electron transport chain and energy transduction systems (114, 115).

Figure 6. Structure of mitochondrial FoF_1 ATPase (complex V).

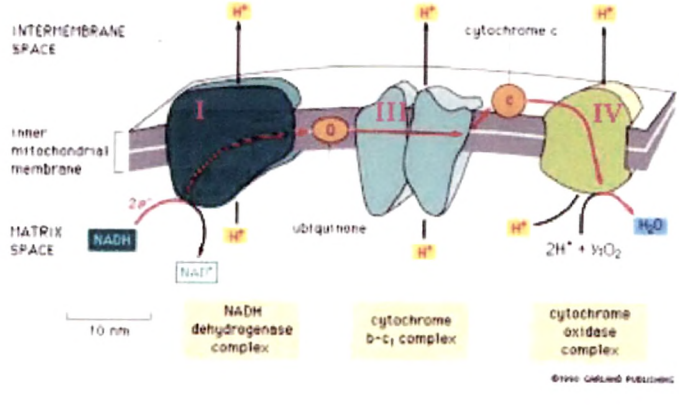


Cytochrome Oxidase

The enzyme cytochrome oxidase is the terminal sink of electrons in the electron transport chain of all aerobic organisms (115). In the higher organisms the cytochrome oxidase complex (complex IV) comprises of 13 polypeptides, two hemes (heme a and heme a_3), two copper atoms (Cu A and Cu B), one Zn and one Mg atoms. Additionally, presence of

one more Cu is also reported (116, 117). The structure and position of cytochrome oxidase in mitochondrial membrane is shown in Fig. 7. Of the thirteen polypeptides three high molecular weight peptides namely viz. COX I, COX II and COX III are mitochondrial gene products and represent the minimum catalytic subunits. The remaining polypeptides are nuclear gene products and are regulatory polypeptides (72).The enzyme exists as dimer deeply embedded in the inner membrane (72). The embedded enzyme is surrounded by core lipids: mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) (118). The enzyme has an absolute requirement for DPG for its activity (119, 120). Since cytochrome oxidase is the terminal electron sink, the rate of respiration in mitochondria depends on cytochrome oxidase content.

Figure 7. Structure and location of cytochrome oxides in mitochondrial membrane



Microsomes

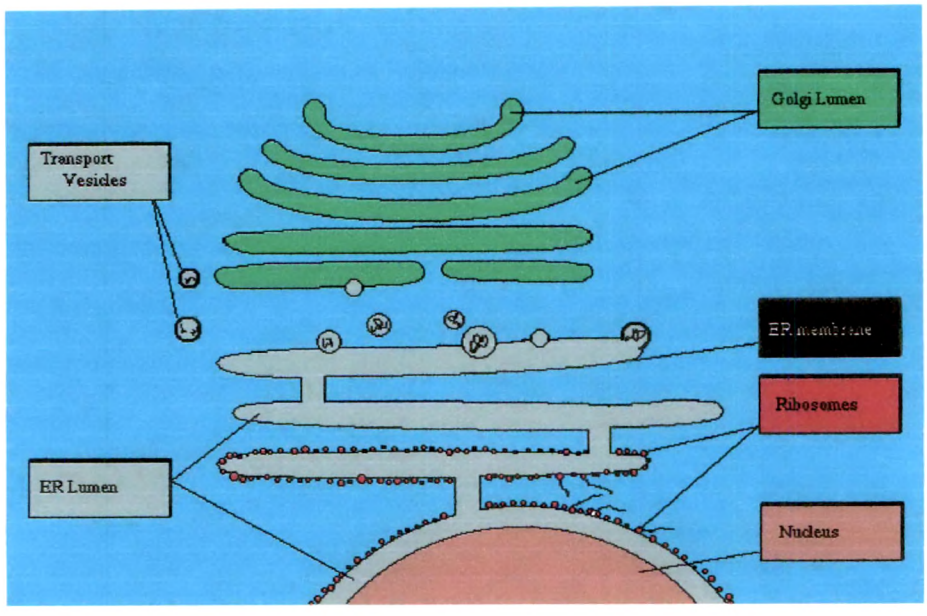
The microsomes are the artefacts of preparation derived from endoplasmic reticulum (ER). The ER is a very amazing part of the cell. It is responsible for a wide range of tasks that includes the biosynthesis of lipids for constructing new membranes, proteins (via ribosomes) and complex carbohydrates. The ER membrane typically makes up more than half of the total membrane in the cell and is located between the nucleus and the cytosol and specifically the golgi apparatus. This means that there are 2 membranes between the nucleus and the Golgi Apparatus, the outside ER membrane and the nuclear membrane (This is because the ER is continuous with the outer nuclear membrane). However, there are 2 membranes between the golgi and the ER and there is a LARGE amount of transfer between the two organelles, which suggests there is probably transport occurring through transport vesicles which is shown schematically in Fig. 8 The ER is made up of two phospholipid bilayer membranes. The enclosed 'sac' is called the ER lumen, the internal space of the ER. The ER is thought to be a single continuous membrane (121).

There are two types of ER:

Rough ER: Is associated with ribosomes (the dots on its boundaries) and the membranes tend to be in 'sheets' or flatten sacs called cisternae.

Smooth ER: Which lacks ribosomes, and is also more of a mesh of smaller interconnecting tubes.

Figure 8. Endoplasmic reticulum

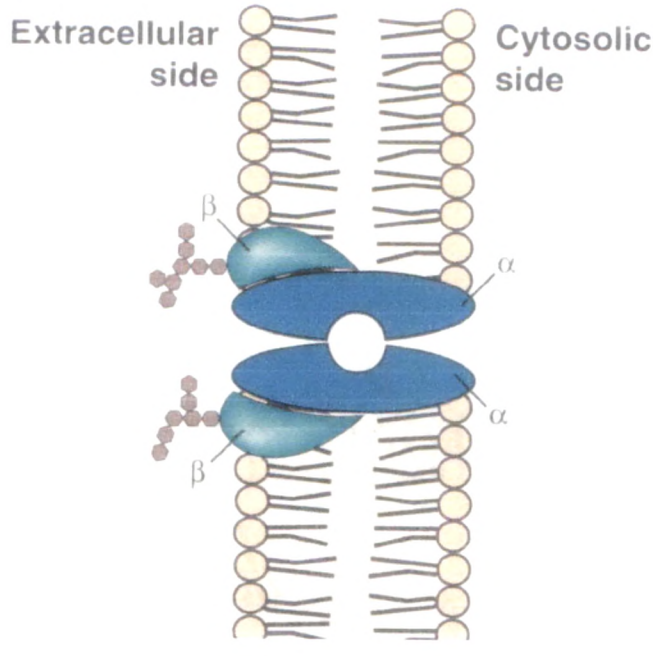


Na^+, K^+- ATPase

The sodium-potassium ATPase (Na^+ K^+ -ATPase or Na^+ K^+-pump) is the ubiquitous plasma membrane enzyme present in the all eukaryotic cells, which actively extrudes Na^+ from cells in exchange for K^+ at a ratio of 3:2 (122, 123). The schematic diagram of Na^+, K^+-ATPase is shown in Fig. 9. It is an oligomer containing two α subunits of about 110 kDa each and two β subunits of about 55 kDa each. Besides, a small hydrophobic protein called the γ-subunit is associated with Na^+, K^+-ATPase and modulates its activity (124). Na^+, K^+ -ATPase have different isoforms depending on tissue type and different function. Four isoform of α-subunit (α_1 α_2 α_3 and α_4) and three isoform of β-subunit (β_1 β_2 and β_3) that are encoded by different genes are found in vertebrates (124-127). Na^+, K^+ -ATPase is known to be deficient in many tissues in diabetic condition (122, 128). Na^+, K^+

-ATPase activity is decreased in red blood cell membranes of type 1 diabetic individual whereas it is normal in the type 2 diabetics (122). Na^+, K^+-pump content is down regulated during diabetic condition which gets up regulated by the insulin treatment (129). Diabetic condition resulted in more than 50 % impairment of Na^+ pump, Ca^{2+}-transport mechanisms and the insulin-dependant glucose transporter GLUT 4 (130). However, not much is known about microsomal Na^+, K^+ -ATPase except that in the heart of diabetic rats, microsomal Na^+, K^+ -ATPase decreased significantly (131).

Figure 9. Schematic diagram of the Na^+, K^+ -ATPase



Glucose-6-phosphatase (G6Pase)

The classical role of Glucose-6-phosphatease (G6Pase) in liver and kidney is production of glucose for release in to blood (132, 133). In liver, G6Pase catalyses the terminal step of glycogenolysis and gluconeogenesis (133). Fig. 10 shows the structure of human G6Pase and Fig. 11 shows the structure-function relationship of the G6Pase system according to the substrate translocase-catalytic unit hypothesis (134). The cross section of endoplasmic reticulum (ER) (Fig. 11) shows T_1 (G6P transporter), $T_1\alpha$, $T_1\beta$ and T_3 the substrate/product transporters and/or auxiliary proteins with the indicated specificity. Catalytic unit is G6Pase embedded within the ER membrane with nine-transmembrane-spanning helical regions. Circles on the inner loops of the catalytic unit indicate amino acid residues comprising the phosphates signature motif (134). The activity of G6Pase is regulated by various hormones, mainly at transcriptional level (132). Insulin suppresses the activity of G6Pase by decreasing the amount of messenger ribonucleic acid (m RNA) of the catalytic subunit (132, 135). Glucagon, via cAMP, and glucocorticoids increase the activity of this enzyme (136, 137). The activity and gene expression of G6Pase was increased in db/db mice despite hyperinsulinemia compared to control db/+m mice (138). G6Pase catalyzed rate-limiting step of glyconeogenesis, and hepatic G6Pase activity is increased in diabetes (132). Abnormally high G6Pase activity in liver was noted in poorly controlled or untreated diabetes mellitus (133).

Figure 10. Structure of human G6Pase

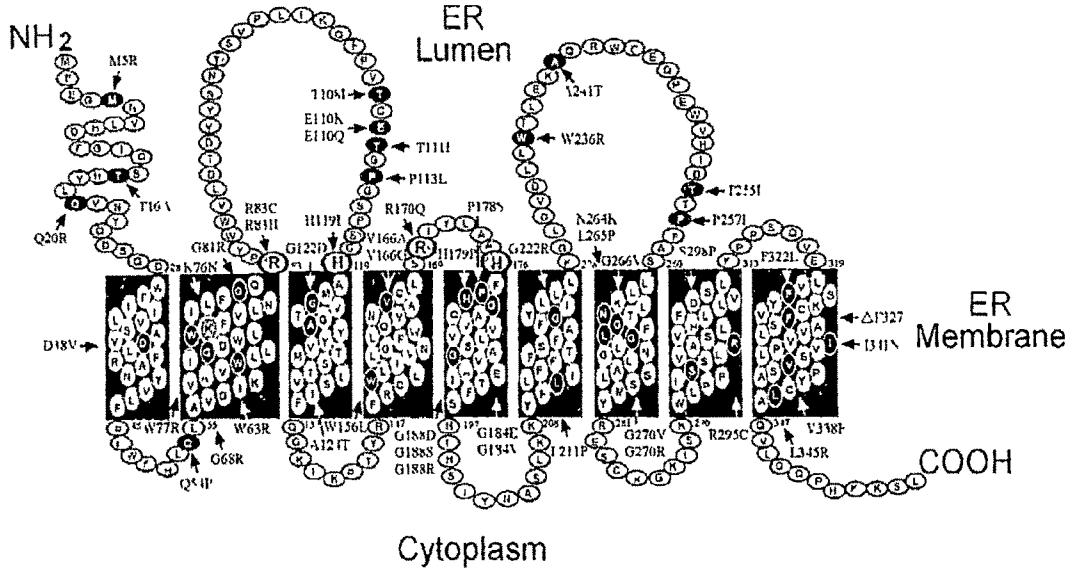
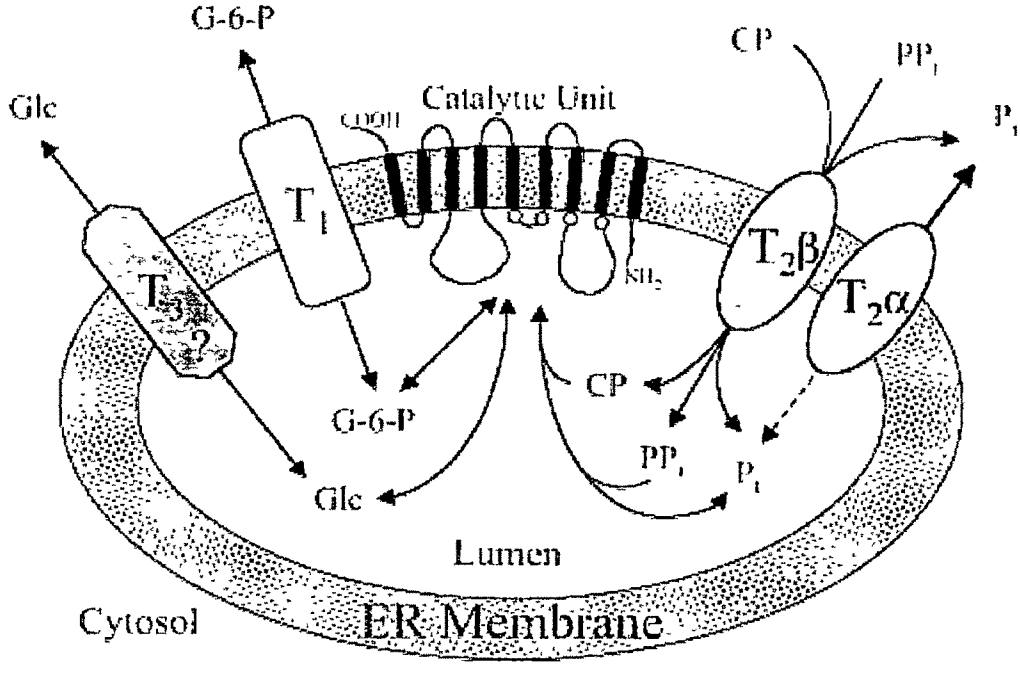


Figure 11. Structure-function relationship of the G6Pase system



Diabetes and membrane structure-function alterations

Hormonal influence on cell membrane is been known (139, 140). One hypothesis postulate that changes in membrane lipid structure and micro-viscosity may play the role of a hormonal information transducer (139, 140). Several major functions of the plasma membrane such as enzyme activities and ligand-receptors interaction depend on membrane fluidity, a concept related to movement of lipids and proteins in the plane of the membrane (141, 142). The mitochondrial and microsomal membrane enzymes are known to have requirement for specific phospholipid classes (118, 131, 143-145) and phospholipid classes and sterols are capable of regulating membrane protein activity (146). Deficiency in phosphatidylserine (PS) is associated with a loss of Na^+, K^+- ATPase activity in several types of cataract (147).

A large number of polypeptide hormones can provoke rapid changes in phospholipid metabolism in their target tissues (148-151). Insulin acutely increases phospholipids in the phosphatidateinositol cycle in rat adipose tissue both In Vivo and In Vitro (150, 152). Thyroid hormones regulate the mitochondrial level of cardiolipin by regulating the activity of cardiolipin synthase (153). Shifts in membrane phospholipid content may be important in regulating the activity of a variety of cellular enzymes (154, 155). Effect of diabetes on liver and kidney plasma membrane phospholipids and phospholipid classes are different (155). Diabetic state resulted in increased cholesterol and total phospholipid contents in reticulocytes whereas in RBC the contents of cholesterol and total phospholipids decreased (156, 157). Fatty acid desaturases decreased in diabetes in liver microsomes (158). Majority of the phospholipid classes except SPM and DPG are

synthesized in the microsomes and transferred to other membrane system (159-161). The latter two are synthesized respectively in the plasma membranes and mitochondria (159-161). Hyperglycemia in diabetes alters the phospholipid transfer protein (PLTP) secretion which in turn affects the lipoprotein metabolism (162, 163). Diabetes resulted in decreased nerve conduction velocity, Na^+, K^+ -ATPase activity and an abnormal phospholipid fatty acid composition (144). Changes in membrane phospholipid and fatty acids as well as decreased activities of membrane-bound enzyme were noted in diabetic rat heart microsomes (131). In diabetes the relative abundance of phosphatidylethanolamine (PE) increased in erythrocyte and polymorphonuclear leukocyte membrane, whereas those of sphingomyelin and phosphatidylcholine (PC) were decreased in platelets and polymorphonuclear leukocyte membrane (164). The percentage of PS was reduced in erythrocyte but increased in platelets (164). Membrane fluidity of platelets and polymorphonuclear leukocyte membrane alters in both type 1 and type 2 diabetes (165). The results thus emphasize the fact that effects of insulin on lipid/phospholipid metabolism are diverse and tissue/system specific.

Free Radicals

It is now being increasingly recognized that the free radicals plays an important role in various human diseases (166-171).

A free radical is a chemical species (any atom, group of atoms or molecules) with one impaired electron occupying an outer orbital.

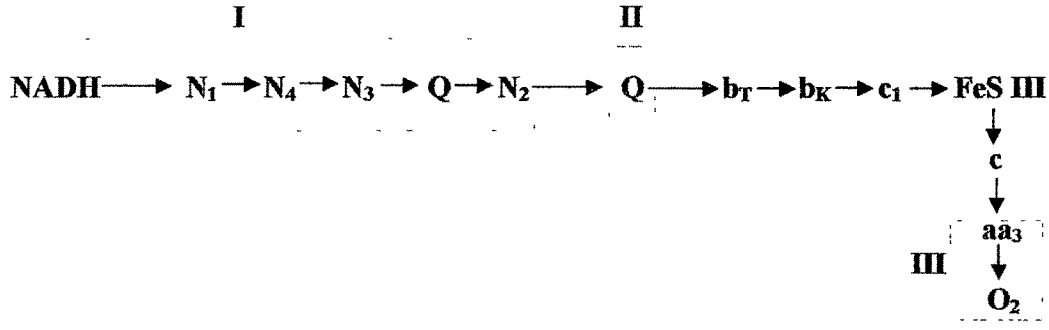
Free radicals broadly have the following properties:

1. High reactivity, 2.Short life span, 3. Autocatalytic and diverse chemical reactivity and 4. Low chemical specificity.

The pivotal compound in the initiation and propagation of free radical reactions is molecular oxygen. The resulting intermediates formed during reduction of molecular oxygen to water are superoxide among radical (O_2•), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). Approximately 1-2 % leakage of these intermediates occurs. Thus, the most important free radical in biological systems is radical derivatives of oxygen (172).

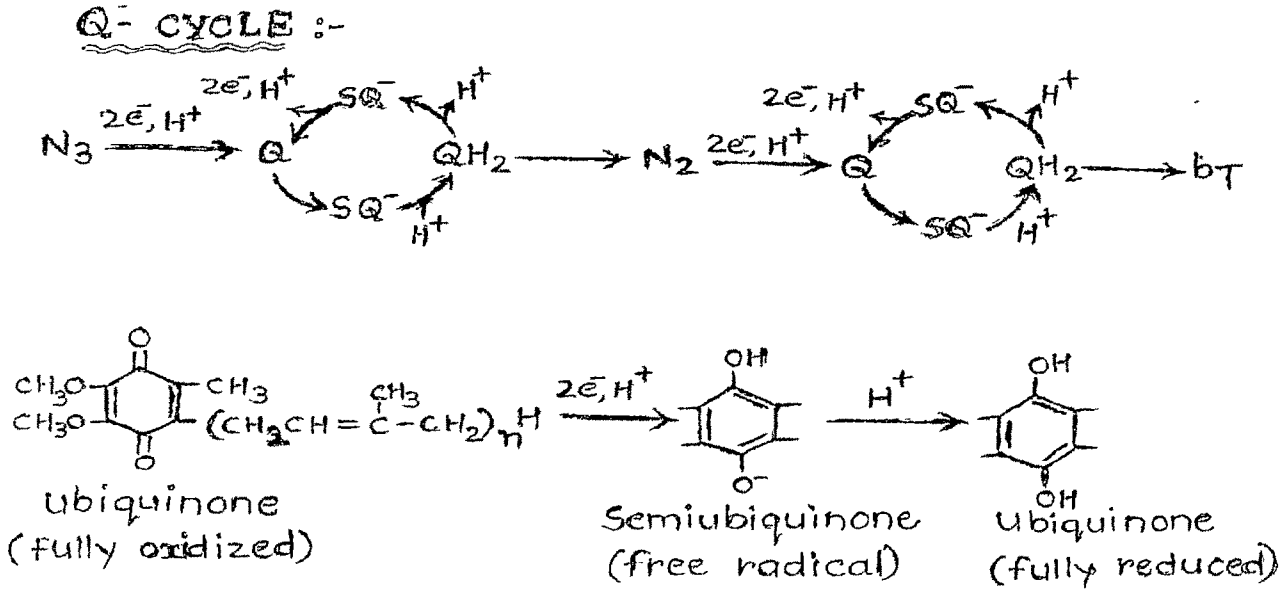
One of the potential sites for the reactive oxygen species (ROS) is mitochondrial electron transport chain (ETC). The schematic presentation and the potential sites for ROS generation in ETC are shown in Fig. 12.

Fig. 12 Electrone transport chain



Sites of ROS generation

I NADH dehydrogenase
II CoQ
III Cytochrome oxidase

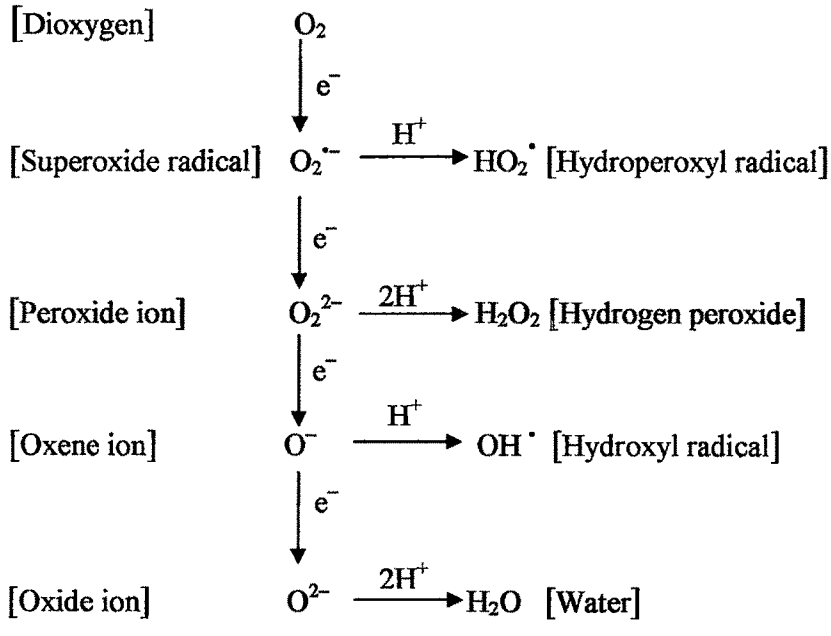


The major oxygen derived free radicals are:

- Singlet oxygen
- Superoxide anion
- Hydrogen peroxide
- Hydroxyl radical
- Peroxyl radical
- Peroxides and lipoperoxides
- Nitric oxide
- Hypochlorous acid
- Other radicals : S-centered; e.g. Thiyl ($RS^{\cdot}$)

The products derived from the oxygen by successive one electron reduction are schematically given Fig. 5.

Figure 5. Products derived from the successive one electron reduction of dioxygen.



The sites of reactive oxygen species (ROS) generation are listed in the Table 8.

Table 8. Sites of ROS Generation in a Cell

Autoxidation of small molecules	→	Thiol Hydroquinone Catecholamines Flavins
Soluble Oxidase Enzyme	→	Xanthene oxidase
Mitochondrial electron transport	→	Electron transport chain
Endoplasmic reticulum and Nuclear membrane transport	→	Mixed function oxidase NADPH oxidase
Peroxisome	→	D-amino acid oxidase Urate oxidase α-hydroxyacid oxidase Fatty acyl CoA oxidase
Plasma membrane	→	Lipooxygenase Cyclooxygenase NADPH oxidase (phagocytes)

The main biological targets of free radical attack are lipids, sulphydryl – containing proteins, carbohydrates and DNA. And the products (biomarkers) of this attack are as given in Table 9.

Table 9 Biomarkers of oxidative stress

ROS and RNS	→	Superoxide radical, hydrogen peroxide, nitric oxide, hypochlorus acid, peroxides, peroxynitrite, singlet oxygen metal-oxo complex, semiquinone radical, heme proteins,
Products of Lipid Peroxidation	→	MDA, 4-HNE, hydroperoxides, conjugated dines, F2-isoprostance dicarboxylic acid
Products of DNA oxidation	→	modified base, 8-oxo-2'-deoxyguanosine, strand breaks
Primary products of protein oxidation	→	o-tyrosine, o,o'-dityrosine, 3-chlorotyrosine, 3- nitrotyrosine, dihydroxyphynylalanine, protein disulfides, methionine sulfoxide, hydroperoxide of isolucine, lucine and valine protein carbonyls-adipic semialdehyde, 2-oxohistidine
Primary products of protein oxidation	→	AGEs: Pentosidine, Crosslines, Vesperlysines ALEs: MDA-Lys, MDA-LDL, HNE-(Lys, His, Cys) Pyrroles EAGLEs: CML, CMA, CEL Argpyrimidine GOLD, MOLD
Antioxidant defense system and total antioxidant status		
Levels of enzymes and antioxidants		

AGEs- advanced glycation end products; ALEs- advanced lipoxidation products; MDA- malondialdehyde; HNE- 4-hydroxynonenal; CML- carboxymethyl-lysine CMA- n-carboxymethylarginine; CEL- carboxyethyl-lysine

Lipids

Peroxidation in polyunsaturated fatty acids (PUFA) in organelles, plasma membranes, causing cross linking and affecting membrane permeability (173)

Carbohydrates

Polysaccharide depolymerization.

DNA

Hydroxyl radical causes base modification, nicking, cross linking, scission of DNA strand. (174)

Biological Antioxidant Defense System

Chemically antioxidant is a compound or a substance that inhibits oxidation. Another definition proposed by Krinsky is "compound that protects biological systems against potentially harmful effects of processes or reactions that can cause excessive oxidation" (175). Antioxidants defend cell against free radical attack by preventing radical formation, intercepting radical from further activity of participating in repair of damage caused by free radical.

Antioxidants can be classified in to two broad categories depending on their mode of action:

1) Preventive inhibitor: Retard the initiation phase of free radical attack.

2) Free radical chain breaking antioxidants: Interrupt the autooxidation chain by reacting with free radicals to produce stable product (176).

Free Radical Scavengers

These include:

Enzymatic

Superoxide dismutase (SOD)

Glutathione peroxidase (GPox)

Catalase

Non- enzymatic

1) Lipid soluble: α –Tochopherol,bilirubin , β- carotene

2) Water soluble: Ascorbic acid, flavinoids, glutathione (GSH)

3) Antioxidant minerals: Cu, Zn, and Mn (SOD), Fe (Catalase) and Se (GPox)

Hormonal

Melatonin

Enzymatic Free Radical Scavengers

This group includes the enzymes, which detoxify free radical and its product

(i) Superoxide dismutase (SOD): EC-1.5.1.1.

SOD specifically catalyzes the dismutation of superoxide anion radical to hydrogen peroxide and oxygen (177).

$$O^{\bullet}_2{}^- + O^{\bullet}_2{}^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

The SOD family consists of four metallo forms containing two copper and zinc, one manganese and one iron (VT 34). In eukaryotes, three forms of SOD are known to exist: Cu-Zn SOD (32 kD) in cytosol. Extracellular SOD (EC-SOD) (135 kD) may function as a scavenger of superoxide produced extracellularly such as from the production of neutrophils or leakage from erythrocytes. Mn SOD (88 kD) is present in mitochondrial matrix.

(ii) Glutathione peroxidase (GPox):

It catalyzes the oxidation of GSH to oxidized glutathione (GSSG) at the expense of hydrogen peroxide (178).

$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$$

$$2GSH + ROOH \longrightarrow GSSG + ROH + 2H_2O$$

GPox exists in two forms: Selenium dependent and selenium independent (a) Selenium dependent GPox (84 kD): EC-1.11.1.9. It has high affinity towards both hydrogen peroxide and organic hydroperoxides. It is found in both cytosol and mitochondria and (b) Selenium independent of GPox (50 kDa): EC-22.5.1.18.

They are the glutathione-s- transferases (GST) and have low activity towards organic hydrogen peroxides and none for hydrogen peroxide. They have multiple functions, but are mainly involved in the biotransformation of xenobiotics and detoxification of carcinogens. Their intracellular distribution is in the cytosol and mitochondria.

Phospholipid hydroperoxide glutathione peroxidase (PLGPox- 20 kD): EC-1.11.1.12. It is the second selenoenzyme discovered in mammals. It is regarded as a cytosolic enzyme that is active on membrane to which it is bound to some extent.

(iii) Catalase (24 kD): EC-1.11.1.6.

It is the oldest known enzyme and catalyzes the reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Most aerobic cells contain this enzyme. In animals it is present in all major body organs, being especially concentrated in liver and erythrocytes. At sub-cellular level it is found in peroxisome (80%) and cytosol (20%). It exists as a tetramer, each monomer containing a heme at the active site. The hydrogen peroxide formed by SOD is decomposed to water and oxygen by catalase and thus it protects the cell from the deleterious effects of hydroxyl radical. Catalase also catalyzes the oxidation of H^+ donors e.g. methanol, ethanol (peroxidic activity)

Non-Enzymatic Free Radical Scavengers

(i) Glutathione (GSH)

Gamma glutamyl cysteinyl glycine or GSH is the most important thiol present in the cell in mM range. It acts as a substrate for several transferases, proxidases and other enzymes that prevent or mitigate the deleterious effects of free radicals by catalyzing oxidation as shown:

$$2GSH \longrightarrow GSSG$$

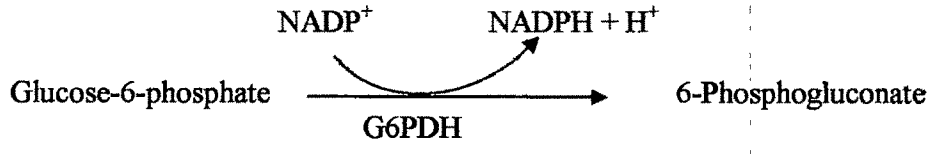
Because changes in GSH status may ultimately reflect the mechanism of toxicity of a compound and to some degree the health status of the biological component under study e.g. cell, blood, tissue, quantitation of GSH status is of interest (178).

Thus, although reactive oxygen species are produced in normal cellular process and have the capacity to cause damage, but are held in check by antioxidant defense systems (172).

Reducing Equivalent Forming System

Glucose 6 phosphate dehydrogenase (G6PDH) (21 KD): EC-1.1.1.49

It is the first enzyme of the hexose monophosphate shunt initializing $NADP^+$ to form reducing equivalent NADPH.



Thus the activity of the enzyme can help to maintain the reducing environment of the cell.

Lipid Peroxidation (LPO)

Polyunsaturated fatty acids (PUFA) are particularly vulnerable to free radical attack. The oxidative damage is termed LIPID PEROXIDATION. It has been broadly defined as "the oxidative deteriorations of polyunsaturated lipids" (179).

Oxidative Stress

Biological system provides favorable condition for uncontrolled oxidative reactions because of the existence of unsaturated fats in cell membrane and the abundance of oxidative reactions in normal metabolism.

Thus, the cell is in equilibrium, balancing between pro-oxidants (oxidizing species) and antioxidants. Oxidative stress occurs when there is a disturbance in this balance in favor of the pro-oxidants.

Oxidative stress implies that:

1) There is a natural balance between free radicals and antioxidant defense in the normal cells.
2) Damage or cell death results when balance is tipped in favor of free radicals.
   (a) Antioxidants are depleted.
   (b) Formation of radical is increased beyond the ability of the defense to cope with them.
3) Free radicals cause non-specific or random cell damage. (180).

ROS and Diabetes

Reactive oxygen species (ROS) have been implicated in diabetes; mitochondria are the major cellular sites of oxygen consumption. However, a small but significant amount of oxygen consumption also occur extra-mitochonmdrially, i.e. in peorxisomes, microsomes and cytosol. Mitochondrial as well as extra–mitochondrial oxygen consumption has implications for ROS generation. The ROS generation in a cell is a spontaneous process.

During respiration under normal physiological conditions there is 2-3 % of electron – slippage which leads to ROS production. Approximately, one cell consumes 10^{13} oxygen molecules per day. Hence, about 2-3 x 10^{11} free radicals are formed per cell per day. It has also been reported that in diabetic condition, hyperglycemia leads to excess production of ROS (28, 29, 181, 182). Superoxide is generated by the process of glucose autoxidation that is associated with the formation of glycated proteins in the plasma of diabetic patients (29). Over production of ROS and reactive nitrogen species (RNS), lowered antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus can contribute to endothelial, vascular and neurovascular dysfunction (181). Clinical and experimental investigations have suggested that in patients with diabetes, increase sympathetic activity, concomitant diabetic autonomic neuropathy, the activated cardiac renin-angiotensin system, myocardial ischemia/functional hypoxia and elevated level of glucose will result in oxidative stress (28), which in turn leads to abnormal gene expression, altered signal transduction and the activation of pathways leading to programmed myocardial cell death which play a critical role in development of diabetic cardiomyopathy (20).

High glucose flux through aldose reductase inhibits the production of antioxidant gene expression (183). High glucose induced mitochondrial ROS production, which suppress first-phase of glucose-induced insulin secretion (GIIS) at least in part, through the suppression of glyceraldehydes 3 phosphate dehydrogenase (GAPDH) activity (27, 171). Hyperglycemia leads to excess production of ROS, lipid peroxidation (LPO) and protein glycation that may impair cellular calcium homeostasis and results in calcium

sequestration and dysfunction in diabetic tissues (182). Increased oxidative stress is believed to play an important role in the etiology and pathogeneses of chronic complications like atherosclerosis, myocardial infarction, hypertension, nephropathy etc (28, 181, 184).

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