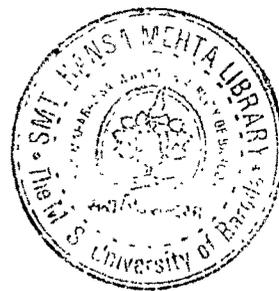
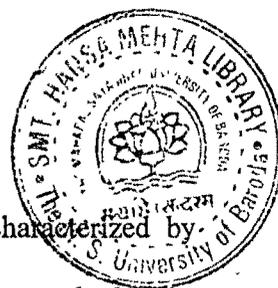


Chapter I

Introduction



- **Diabetes mellitus**
- **Insulin**
- **Consequences of insulin deficiency/ resistance (Diabetes mellitus)**
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Introduction

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. Over 20 million people are affected by diabetes in India. These numbers are expected to increase to 57 million by 2025. In the 1970s, the prevalence of diabetes among urban Indians was reported to be 2.1 per cent and this has now risen to 12.1 per cent (Pradeepa and Mohan, 2002). People with diabetes are 25 times more likely to develop blindness, 17 times more likely to develop kidney disease, 30-40 times more likely to undergo amputation, two to four times more likely to develop myocardial infarction and twice as likely to suffer a stroke than non-diabetics (Pradeepa et.al., 2002).

History

Diabetes has been recognized since ancient times. In 1679 the discovery that the urine of a diabetic person had a sweet taste, gave the condition its name. The term "diabetes mellitus" was derived from 2 terms : The Greek word *Diabetes* = to Siphon /pass through; and the Latin word *mellitus* = sweet as honey

A study of the ancient literature indicates that Diabetes was fairly well known and well conceived as an entity in ancient India. Its earliest reference (1000 BC in the Ayurvedic literature) is found in mythological form where it is said to have originated by eating Havisha, (Charak Samhita, 1977), a special food which used to be offered at the

times of yagna organized by Dakshaprajapati. The disease was known as 'Asrava' during vedic era (6000 BC) and a detailed description of it is available in Brahattraiz viz. Charak Samhita, Sushruta Samhita and Vagbhata. Asthanga Haridaya (600 AD) is the first medical treatise in which one gets clear definition of madhumeha/diabetes mellitus by mentioning glycosuria (madhviv mehati-honey like urine). The aetiology, pathogenesis and the principles of management, which are described in Ayurvedic classics, resemble with the modern concepts almost in toto. Ayurvedic medicine emerged during the rise of the philosophies of the Upanishads, Buddhism, and other schools of thought in India. Herbs played an important role in Ayurvedic medicine. The principal Ayurvedic book on internal medicine, the Characka Samhita, describes 582 herbs.

To enumerate, earliest known record of diabetes (1552 B.C.) was mentioned on 3rd dynasty Egyptian Papyrus by physician Hesy – Ra; mentioning polyuria as a symptom.

1st Century A.D. – Arateus describes diabetes as “the melting down of flesh and limbs into urine”.

Early 19th Century – First chemical tests developed to indicate and measure the presence of sugar in the urine.

In 1869 – Paul Langerhans, a German medical student, announces in a dissertation that the pancreas contains two systems of cells. One set secretes the normal pancreatic juice, the function of the other was unknown. Several years later, these cells are identified as the 'islets of Langerhans.'

During 1910 – 1920 – Frederick Madison Allen and Elliot P. Joslin emerge as the two leading diabetes specialists in the United States. Joslin believes diabetes to be 'the

best of the chronic diseases' because it was 'clean, seldom unsightly, not contagious, often painless and susceptible to treatment.'

In 1921 – Frederick Banting and Charles Best at the University of Toronto, isolated insulin from pancreas and proved that it could control hyperglycemia and glycosuria in experimental diabetic dogs.

In 1926 – J. J. Abel first crystallized Insulin. This incident raised the great controversy regarding bioactivity of crystallized insulin.

In 1959 – Insulin was the first protein whose entire primary structure was identified by Sir Frederic Sanger.

In 1982 – Recombinant DNA insulin was introduced and enzymatic conversion of pork insulin sequence to human insulin sequence developed.

Classification

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (World Health Organization, 1980), and, in modified form, in 1985 (World Health Organization, 1985). The 1985 classification was widely accepted and used internationally which includes both staging of diabetes mellitus based on clinical descriptive criteria and a complimentary aetiological classification. Diabetes mellitus is typically classified into two main subtypes: type-I or insulin-dependent diabetes (IDDM), and type-II or non-insulin-dependent diabetes (NIDDM).

Type 1 diabetes (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin primarily due to pancreatic islet beta cell destruction and more prone to ketoacidosis, for which neither an aetiology nor a pathogenesis is known

(idiopathic). This form develops most frequently in children and adolescents, but is being increasingly noted later in life and are attributable to an autoimmune process.

Type 2 diabetes (formerly named non-insulin-dependent) which results from defect(s) in insulin secretion almost always with a major contribution from insulin resistance, the body's inability to respond properly to the action of insulin. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly familial, but it is only recently that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors.

People with Type 1 diabetes are usually totally dependent on insulin injections for survival. Such people require daily administration of insulin. The majority of people suffering from diabetes have the Type 2 form. Although they do not depend on insulin for survival, about one third of sufferers needs insulin for reducing their blood glucose levels.

Other forms of diabetes include (WHO and NDDG classification) :

- Gestational diabetes.
- Genetic defects of β cell function.
- Genetic defects in insulin action.
- Diseases of the exocrine pancreas.
- Endocrinopathies.
- Drug or chemical induced.

- Infections.
- Uncommon forms of immune mediated diabetes.
- Other genetic syndromes sometimes accompanied with diabetes.

Diabetes in pregnancy may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of prenatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers.

Diagnostic criteria for diabetes mellitus

Fasting plasma sugar (FPS), Postprandial plasma sugar (PP₂PS) and oral glucose tolerance test (OGTT) are the golden criteria for the diagnosis of diabetes mellitus. Generally FPS after overnight fasting and PP₂PS, two hour after a meal is routinely checked for the diagnosis. OGTT is recommended in case where person is not frank diabetic.

Recently new classification and diagnostic criteria for diabetes were proposed by the American Diabetes Association (ADA), WHO and Japan Diabetes Society (JDS) between 1997 and 1999. Diabetes is classified in to four etiological categories; type 1, type 2, diabetes due to other specific mechanism or conditions and gestational diabetes. Following plasma glucose levels [fasting plasma glucose (FPS) 2-h plasma glucose in the 75g oral glucose tolerance test (2-hPG)] been suggested for the diagnosis of diabetes:

Normal type - FPS < 6.1 mmol / L (110 mg/dl) and 2-hBG < 7.7 mmol/L(140 mg/dl)

Borderline type - FPS > 6.1 mmol / L (110 mg/dl) or = < 7.0 mmol / L (126 mg/dl)

PP₂PS > 7.7 mmol/L(140mg/dl) or = < 11.1 mmol / L (200 mg/dl)

Diabetic type - FPS > 7.0 mmol / L (126 mg/dl) and PP₂PS > 11.1 mmol/L(200 mg/dl)

Borderline corresponds to the sum of impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) based on ADA and WHO criteria.

Prognostic criteria - Prognosis of the disease is monitored by conducting following test
FPS > 7.0 mmol/L (126 mg/dl) and PP2PS > 11.1 mmol/L (200 mg/dl) were recommended as high risk values for hyperglycemia.

Glycosylated Hb levels provides a time averaged picture of the patient's blood glucose concentration over the past 3 months.

Decreased blood pH - < 7.4 is the indicators of acidosis.

ketone body in serum > 1mEq/L can lead to acetone breath and ketoacidosis.

Microalbuminuria and proteinuria are the indicators of diabetic nephropathy.

Diabetic retinopathy is detected clinically by presence of visible ophthalmoscopic retinal microvascular lesions and estimation of Advanced Glycated Endproducts (AGEs).

Diabetic animal models

Study of multifactorial genetics of diabetes is feasible due to availability of various types of genetically diabetic animal models. Animal models also provide unique opportunities for investigating the toxicity and efficiency of therapeutic measures developed for prevention and cure of diabetes and its complications. A large array of animal models are available for experimentation relevant to the study of diabetes especially the rats or mice like db/db mouse (obese rodent with severe diabetes), ob/ob mouse (obese rodent with mild diabetes), sand rat (nutrition induced diabetes), GK rats (Goto-kakizaki – diabetes developed by selective inbreeding), NOD mouse (Non-obese diabetic – spontaneous diabetes), BB rats (Biobreeding rats – spontaneous diabetes) and experimentally induced diabetic rats/mice by chemical diabetogens like alloxan and

streptozotocin.. All these animals can be broadly classified into two types, one where syndromes resemble to NIDDM and another type where syndromes resemble to IDDM. In present study experimentally induced diabetic rat models were used. Alloxan was used as diabetogen, as it's known cause selective β cells destruction by generating ROS (Munday, 1988; Malaisse, 1982). Low affinity glucose transporter GLUT2 and glucokinase makes β -cells susceptible for the alloxan toxicity (Elsner et. al., 2002). This alloxan induced diabetic rats show symptoms more like IDDM. The degree of β -cell destruction depends upon the dose, which is administered to induce diabetes in rats. Moreover, atherogenic rat models by cholesterol feeding is also widely used to investigate the effects of dyslipidemia (Gao et.al., 2002). Effects of dietary fats include the development of arteriosclerosis in humans and experimental animals, in addition to hypercholesterolemia (Imaizumi et.al., 2000).

Insulin

Insulin is the major hormonal regulator of glucose metabolism. The human insulin gene is located in region p13 of the short arm of chromosome 11, adjacent to the genes for insulin-like growth factor II (IGF – II) and tyrosine hydroxylase (Bell et.al., 1980). In mammals, insulin gene expression and biosynthesis are restricted to the β cells of the endocrine pancreas, with the possible exception of the yolk sac and fetal liver (Muglia and Locker, 1984). In addition to tissue selective expression, the insulin gene is subject to environmental regulation within the β cell. For secretion, glucose (and cAMP) is a major stimulus of insulin biosynthesis.

Insulin gene has two introns and three exons. Insulin is synthesised as a large precursor molecule i.e. preproinsulin of 15 KDa. Preproinsulin has 23 amino acid long

leader sequence which is cleaved before entering the rough endoplasmic reticulum (RER) and forms the proinsulin of 9 KDa. Inside RER, proinsulin attains a three dimensional structure by forming disulfide linkages between Cys A7 & Cys B7, Cys A20 & Cys B19 and Cys A6 & Cys A11. Proinsulin is transferred into secretory vesicle, where it is stored in the form of zinc hexamers. When there is stimulus for insulin secretion, these vesicles are translocated towards the plasma membrane. During translocation proinsulin is cleaved into insulin and C – peptide by the enzyme carboxypeptidase A. C- peptide does not have biological activity but it is secreted in equimolar quantity along with insulin.

Insulin was the first protein to have its entire primary sequence determined. All known insulins are composed of two polypeptide chains that are linked to one another by disulfide bonds. The A – and B – chains of human, porcine and bovine insulins, like most other vertebrate insulins are composed of 21 and 30 amino acids respectively. These two peptide chains are covalently linked to one another by two cysteine disulfides, one between CysA7 and CysB7 and the other between CysA20 and CysB19. An additional intrachain disulfide connects cysteines A6 and A11. There is only a single difference between the sequences of human and porcine insulins, at position B30, where human insulin has a threonine and porcine insulin has an alanine. Bovine insulin differs from human insulin at three positions. Like porcine insulin. Bovine insulin has an alanine at the B30 position. In addition, threonine and isoleucine at positions A8 and A10 of human insulin are replaced by alanine and valine in the bovine sequence. They have no apparent effect on biologic activity, although they do affect solubility.

Insulin secretion

Insulin secretion depends not only on the ambient concentration of glucose but also on the rate of change of this concentration. When the glucose level increases slowly, the rate of insulin secretion increases in parallel. However, when the concentration of glucose is abruptly increased and then maintained at a high level, insulin secretion follows a biphasic time course. A rapid peak (first phase) is followed by a nadir and a slowly rising second phase. In isolated rat islets or in the perfused rat pancreas, the threshold extracellular glucose concentration is around 5 to 6 mM, half maximal and maximal responses are observed at 9 to 11 mM and 15 to 20 mM, respectively. Glucose enters β cells by facilitated diffusion, through a high K_m (~ 17 mM) transporter that is structurally and functionally similar to the low-affinity glucose transporter (GLUT 2) present in liver cells (Johnson et al., 1990). Glycolysis is the major pathway of glucose metabolism in β cells leading to the rise in ATP/ADP ratio. β -cell possesses a unique signal transduction system which requires metabolism of fuel (glucose or other nutrients) stimulus to initiate insulin secretion. Previously insulin secretion was thought due to a rise in ATP which closes the K^+ -ATP channel resulting in depolarization of the β -cell, activation of voltage gated Ca^{2+} channels and a rise in intracellular Ca^{2+} . In recent years it has become apparent that second messengers and factors like variation in ADP, anaplerosis (replenishment of citric acid cycle with intermediates), a shift from fatty acid oxidation to esterification etc. other than ATP, metabolically sensitive K^+ -ATP channel and Ca^{2+} play essential role in nutrient induced insulin release. Incretin hormones like GLP-1 and GIP are secreted from gut mucosa after food ingestion are known to potentiate insulin secretion via glucose dependent pathway. Incretin binds to its receptor

and activates adenylate cyclase activity, which increases protein kinase A (PKA) activity. Increased PKA phosphorylates K^+ -ATP channel and thereby closes channels leading to increased membrane depolarization and eventually causing more insulin release from β -cells. Some of the recent studies have shown another pathway of insulin release where Ca^{2+} dependent exocytosis of insulin granules is potentiated by a K^+ -ATP channel-independent action of glucose (Fig I).

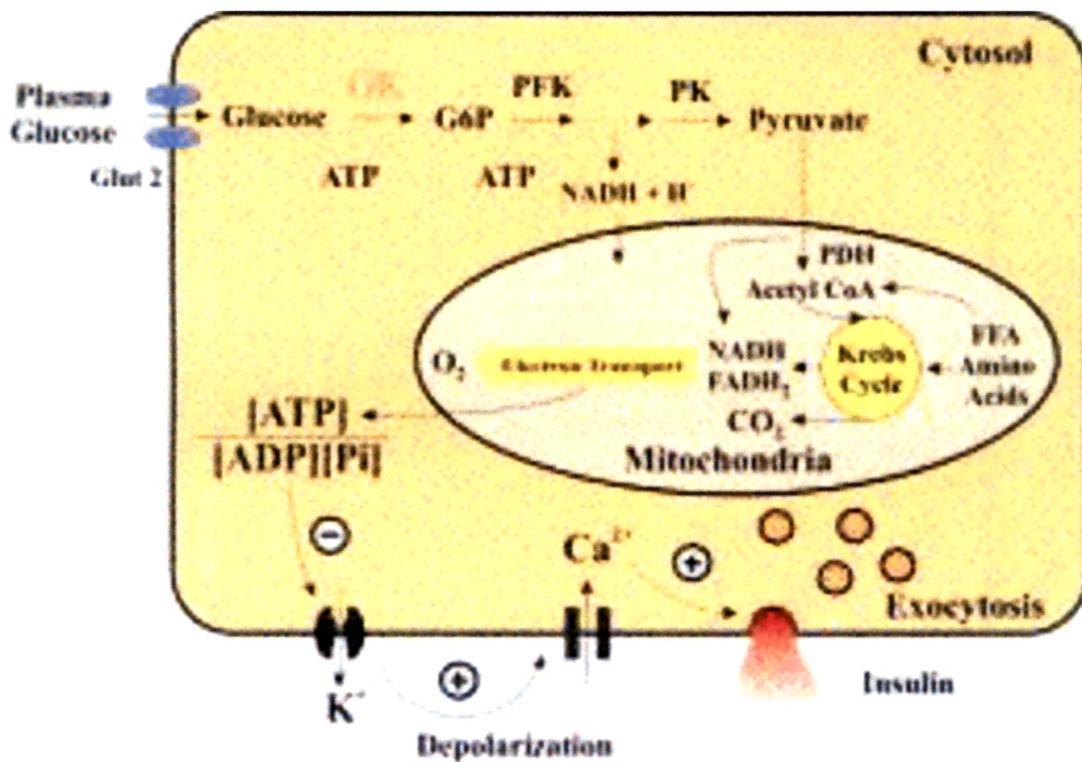


Fig I : Mechanism of insulin secretion from pancreatic beta cells.

Biological effects of Insulin

Muscle, liver and fat are the most important target tissues for insulin with respect to glucose homeostasis. Insulin activates the transport systems and enzymes involved in intracellular utilization and storage of glucose, amino acids, fatty acids, while inhibiting gluconeogenesis and catabolic processes evoked by counter regulatory hormones,

including the breakdown of glycogen, fat and protein. Insulin evokes immediate, intermediate and long term effects on cellular metabolism (Table I). Insulin stimulates rapid phosphorylation of enzymes thought to mediate insulin signals such as raf-I kinase, mitogen-activated protein (MAP) kinase, the S6 kinase, and protein phosphatase-I (Blenis, 1991). Certain enzymes controlling the rate limiting metabolic steps are either activated (pyruvate dehydrogenase, acetyl CoA carboxylase, glycogen synthase) or inactivated (triacylglycerol lipase, phosphorylase kinase, glycogen phosphorylase). Various metabolic enzymes are induced during insulin stimulation, including pyruvate kinase, malic enzyme, glucokinase, whereas others such as phosphoenolpyruvate carboxy kinase (PEPCK), carbamoyl phosphate synthetase – I (CPS-I) and fructose 1,6-bisphosphate are inhibited (O'Brien and Granner, 1991). The long term effects of insulin, which require many hours to several days, include insulin stimulation of DNA synthesis, cell proliferation and cell differentiation; including induction of transcription factors such as *srf* (serum response factor), *c-fos*, *egr-1* (early growth response gene), *c-jun* and *c-myc* (O'Brien and Granner, 1991).

Table I : Biological effects of insulin

Rapid effects –	Tissue	Molecular mechanism
↑ membrane transport of glucose. ↑ membrane transport of amino acids.	muscle, adipose muscle, adipose, liver	PI3K activation
Intermediate effects – <i>Carbohydrate metabolism</i> ↑ glycogen synthesis ↓ glycogenolysis ↑ glycolysis ↓ gluconeogenesis <i>Lipid metabolism</i> ↑ Lipogenesis ↑ esterification ↑ cholesterol synthesis ↓ lipolysis ↓ ketogenesis ↓ fatty acid oxidation ↑ utilization of dietary lipid <i>Protein metabolism</i> ↑ protein synthesis ↓ protein catabolism	muscle, liver muscle, liver muscle, liver, adipose liver Liver, adipose Liver, adipose Liver Adipose Liver Liver, adipose Liver, adipose Liver, muscle, adipose Liver, muscle	↓GSK 3, ↑glycogen synthase, ↑PDH, ↑phosphatase, ↑fructose-6-phosphate-2-kinase and ↓PEPCK ↓ triglyceride lipase, ↑ acetyl-CoA carboxylase, ↑lipoprotein lipase, ↑diacylglycerol acyltransferase, ↑ hydroxymethyl glutaryl-Co-A reductase Activation of transcription and translation factors by phosphorylation (c-jun, c-fos, p70rsk, p90rsk)
Long term effects – ↑ cell growth ↑ cell division ↑ DNA synthesis ↑ RNA synthesis	All the tissues	

Insulin receptor

Insulin receptor is a tyrosine kinase type of receptor. It is a hetero-tetrameric protein containing two α - and two β -subunits. These subunits are linked together by disulfide linkages. Subunit α has insulin binding site and subunit β has ATP binding site, autophosphorylation sites and tyrosine kinase activity domain. Binding of insulin to α subunit of IR leads to activation of autophosphorylation of β subunit and tyrosine kinase activity. This activated tyrosine kinase can phosphorylates several other downstream signaling molecules to mediate the insulin action. Subunit α is regulatory subunit. N terminal of α -subunit is required for ligand recognition and for high affinity ligand binding and C-terminal is involved in covalent interaction with N-terminal of β -subunit and signal transmission from α -subunit to β -subunit. Partial removal of α -subunit by digestion with trypsin or *in vitro* mutagenesis activates tyrosine kinase activity of the β -subunit. Intra cellular domain of β -subunit has tyrosine kinase activity which is prerequisite for insulin action. *In-vitro* mutagenesis experiments have shown that Lys-1030 at ATP binding site of β -subunit is required for insulin action.

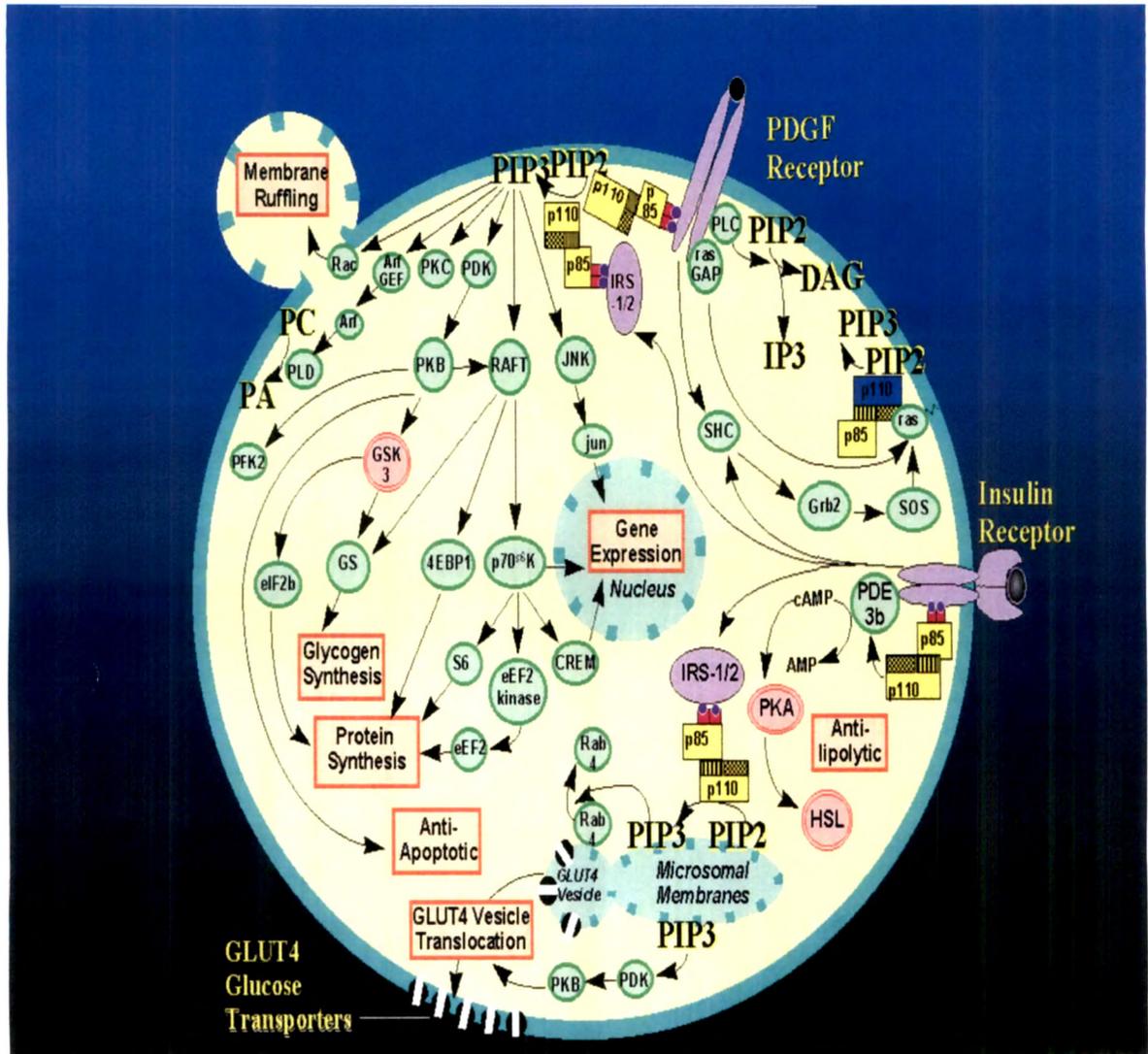
Insulin signaling (Fig 2)

The tyrosine kinase of the insulin receptor is initially stimulated by insulin binding and then greatly augmented by insulin-stimulated autophosphorylation (Wilden et.al., 1992; Rosen et.al, 1983). Activated IR kinase phosphorylates substrates like Shc, IRS-1, IRS-2 and Gab-1 on Tyr residue and provide a docking interface with downstream substrates. IRS contains multiple Tyr phosphorylation motifs that serves as docking sites for SH-2 domain containing proteins like p85 α , regulatory subunit of phosphatidylinositol 3-kinase (PI3K), Grb2, Nck, Crk, Fyn, SHP-2 etc which mediate the

genomic and non-genomic action of insulin. IR signaling involves two major pathways—the mitogen activated protein kinase (MAPK) and the PI3K. Although these pathways are described in linear fashion, each can activate other pathway under certain circumstances. Binding of Grb2 to phosphorylated Shc or IRS via its SH2 domain activates MAP kinase pathway. Grb2 is prebound to mSOS, a nucleotide exchange protein of GDP for GTP on Ras and thereby activate Ras. Activated Ras bind with Raf and recruit it to plasma membrane. Ras-Raf interaction displaces 14-3-3 proteins, which are bound to Raf, and allows phosphorylation of Raf by number of Ser/Thr kinases. Raf-1 activates a dual-specificity kinase, MEK1, by phosphorylating two regulatory Ser residues. MEK1 (also known as MAPKK1) activate extracellular signal-regulated kinases (ERK-1 and ERK-2) by phosphorylating regulatory Ser/Thr residues. Activated ERK mediate the growth promoting effects of insulin by phosphorylating transcription factors such as *elk-1*, *c-jun*, *c-fos* etc.

PI3k gets activated upon binding with IRS and results in production of phosphatidylinositol 3,4,5 phosphate (PIP₃). Activation of PI3-kinase by IRS-1 results in translocation of glucose transporter (GLUT 4) to the cell membrane. PIP₃ binds to the PH domain of PI3-K – dependent kinase (PDK1) and Akt (PK-B). PIP₃ binding leads to activation of PDK-1, which in turn phosphorylate and activate Akt. Akt has been implicated in GLUT 4 translocation and stimulation of glycogen synthesis via inhibiting GSK-3. GSK-3 also inhibits activity of transcription elongation factor, eIF2B by phosphorylating its ϵ - subunit at Ser-540 and thereby inhibits protein synthesis. Activation of Akt via insulin reverses this process and increases the protein synthesis.

Fig 2: Insulin Signalling.



Diabetes mellitus – consequence of insulin deficiency/resistance

The defects in insulin deficiency or insulin resistance leads to hyperglycemia – the hallmark of the X syndrome i.e. diabetes mellitus. As β cell destruction progresses in case of IDDM, plasma insulin levels fall even during the fasted state, hepatic glucose production increases, and the patient requires insulin therapy (Eisenbarth et.al., 1987). With more severe insulin deficiency, plasma FFA levels increase in response to enhanced lipolysis and plasma triglyceride levels may increase because of a decrease in lipoprotein lipase activity (Ong and Kern, 1989). Deficiency of insulin and/or increase in counter-insulin hormones are sufficiently severe to increase glycogen, protein and lipid catabolism; leading to elevated plasma FFA and ketone body levels. Whereas, an earlier abnormality in NIDDM is hyperinsulinemia associated with insulin resistance (Warram et.al., 1990). Patients with type II diabetes and obesity have been shown to have a significant defect in glucose uptake in skeletal muscle (DeFronzo, 1992), a decrease in muscle glycogen synthesis (DeFronzo et.al., 1985), and an increase in lactate production (Bogardus et.al., 1984). Such inhibition of glycogen synthesis cause an increase in glucose-6-phosphate, suggesting a defect in glucose uptake or hexokinase step. Decrease in pyruvate dehydrogenase activity is also seen contributing to the decrease in glucose oxidation and increase in muscle lactate release (Mandarino et.al., 1986). Hyperglycemia may worsen insulin resistance leading to “glucose toxicity” (Rossetti et.al., 1990). Insulin has a profound effect on protein turnover through its dual role as a stimulator of protein synthesis and an inhibitor of protein degradation. In conditions where insulin is lacking, total body protein is lost, particularly evident as wasting of muscle. Hence any failure in insulin function leads to impaired carbohydrate, fat and protein metabolism. As a result,

elevated levels of glucose in the plasma, free fatty acids, triglycerides, cholesterol, VLDL, ketone bodies, etc. are encountered. All these consequences leads to oxidative stress and the accompanied diabetic complications.

Oxidative stress and diabetes

Hyperglycemia is a major risk factor for the development of diabetic microvascular complications (DCCT, 1993) and it promotes many functional changes in the microvasculature that lead to structural tissue changes. The mechanisms that mediate the adverse effects of hyperglycemia include extracellular nonenzymatic glycation processes, sorbitol accumulation through aberrant aldose-reductase enzyme activation and alterations of various signal pathways like diacylglycerol (DAG) – protein kinase C (PKC) pathway (Fig 3). In the presence of a high glucose concentration, glucose can be incorporated nonenzymatically into proteins by an unregulated glycation reaction. The reaction involves the formation of a Schiff base (aldimine), followed by a much slower internal shift, the Amadori rearrangement (Bunn, 1981) (Fig 4). Lysine and valine are the primary sites of glucose addition. Such unregulated glycation changes the protein structure and impairs function. The molecular mechanism of biological oxidation by glucose was first identified in 1912 by Louis Maillard. This French chemist described a brown colour that formed from heating solutions of carbohydrates and amines and termed this process the “réaction du Maillard”. Non-enzymatically glycated proteins slowly form fluorescent cross-linked protein adducts called advanced glycation end products (AGEs). This process known as “browning” or *Maillard reaction* is accelerated by elevation of the ambient glucose concentration. They cause tissue damage because of their reactivity and cross-linking. A prime cross linking intermediate is 3 – deoxyglucosone, which can be

derived from glucose or fructose (Yamada et.al., 1994). One of the AGEs that results from the reaction of pentoses with proteins is pentosidine, a marker of accelerated tissue modification that is enhanced in diabetic patients with severe complications (Sell et.al., 1992). Dyer et.al. treated the rate of accumulation of glycoxidation products in skin collagen as the second-order product of the degree of hyperglycemia and the status of oxidative stress. However, levels of AGEs appear to increase in concert in kidney, vascular tissue, and skin of diabetic animals within only a few weeks after induction of diabetes in animal models (Baynes, 1991), suggesting that AGEs are formed at an early stage in the disease process and that the increase in their levels is systemic. AGE-proteins are chemically damaged proteins. Therefore, it seems likely that, as with other damaged molecules, such as oxidized DNA or lipoproteins, biological mechanisms would have evolved for their recognition and turnover. Endothelial cell AGE receptors internalize AGEs to the subepithelium, thereby enhancing permeability and endothelium-dependent coagulant activity (Vlassara et.al, 1992). AGEs on the surface of diabetic erythrocytes may enhance their interaction with endothelial cells, causing binding and oxidant stress (Fu et.al, 1994), which are important for the development of vascular complications (Nathan, 1993) A number of cell-surface AGE receptors have now been identified and are proposed to have a role in the uptake and catabolism of AGE-proteins in plasma, erythrocyte membranes, and the extracellular matrix. The best characterized among these are RAGE (receptor for AGE) (Schmidt, 1996) composed of R1, R2, and R3 (Li et.al., 1996; Vlassara et.al., 1994) and the macrophage scavenger receptor as CD-36 (Ohgami et.al., 2001) and SCR-II (Takata et.al., 1988; Araki et.al., 1995). RAGE is widely distributed among cell types, including endothelial and smooth muscle cells and

macrophages. AGE-R1 and -R3, components of the AGE-receptor complex, are largely responsible for AGE-recognition and high-affinity binding (Li et.al., 1996). AGE-R2 is subject to AGE-induced phosphorylation (Stitt et.al., 1997). This which plays a role in signal transduction and cell activation associated with AGE-receptor binding (Vlassara et.al., 1989). RAGE, a multiligand member of the immunoglobulin superfamily, is viewed increasingly as an intracellular signal- transducing or pro-inflammatory peptide. In this regard, RAGE may thus be more accurately classified in the family of oxidant-stress-inducing signaling molecules or co-factors. It also is implicated in the transmission of oxidative stress to receptor bearing cells, since binding of AGE-proteins to RAGE on cell surfaces induces an intracellular oxidative stress response in vitro, characterized by increased NF-B, and tissue-factor expression (Bierhaus, 1997). Beside substrate and oxidative stress, there is an alternative explanation for the increase in chemical modification of proteins in diabetes, uremia, and other diseases, i.e., carbonyl stress, which is caused by a generalized increase in the concentration of reactive carbonyl precursors of AGEs, glycoxidation and lipoxidation products. The concentration of the AGE precursor 3-DG, for example, is increased in both diabetic and uremic plasma (Yamada et.al., 1994), and 3-DG-arginine (imidazolone) adducts are increased in blood and tissue proteins in diabetes, in association with nephropathy (Niwa et.al., 1997). However, 3-DG is formed by a nonoxidative rearrangement and hydrolysis, rather than oxidation, of Amadori adducts (Ledl and Schleicher, 1990), or by nonoxidative elimination of phosphate from fructose-3-phosphate (Szwergold and Kappler, 1990).

Fig 3: How too much glucose may lead to long term complications of diabetes

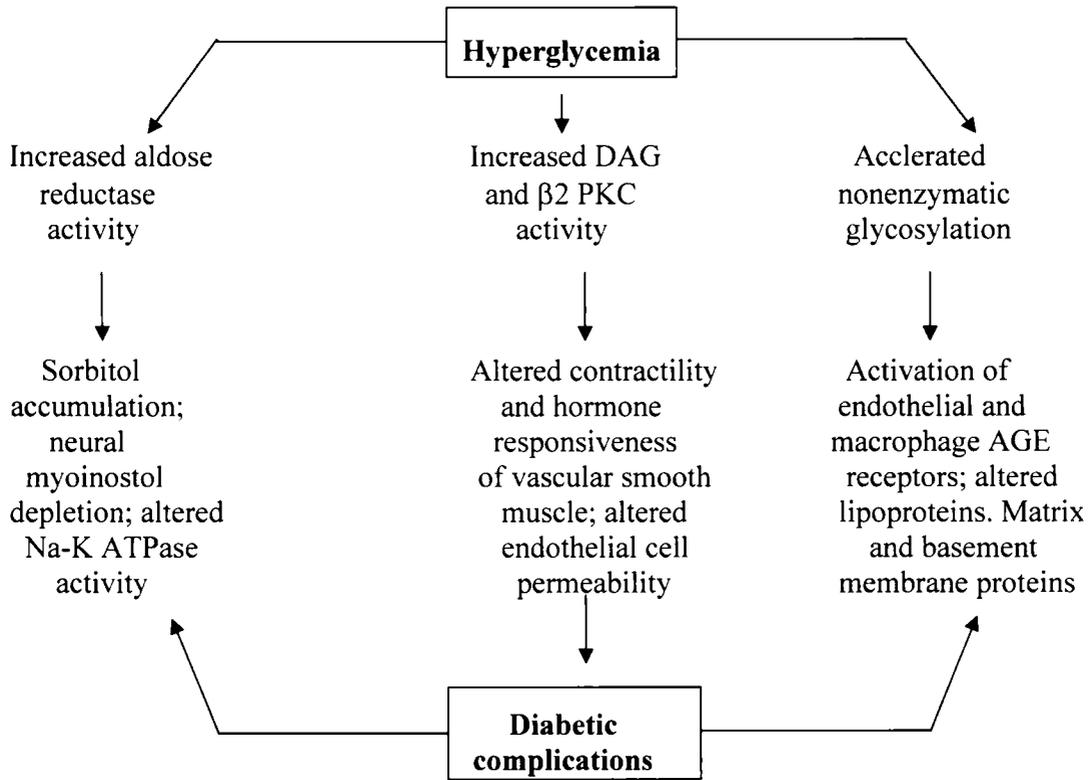
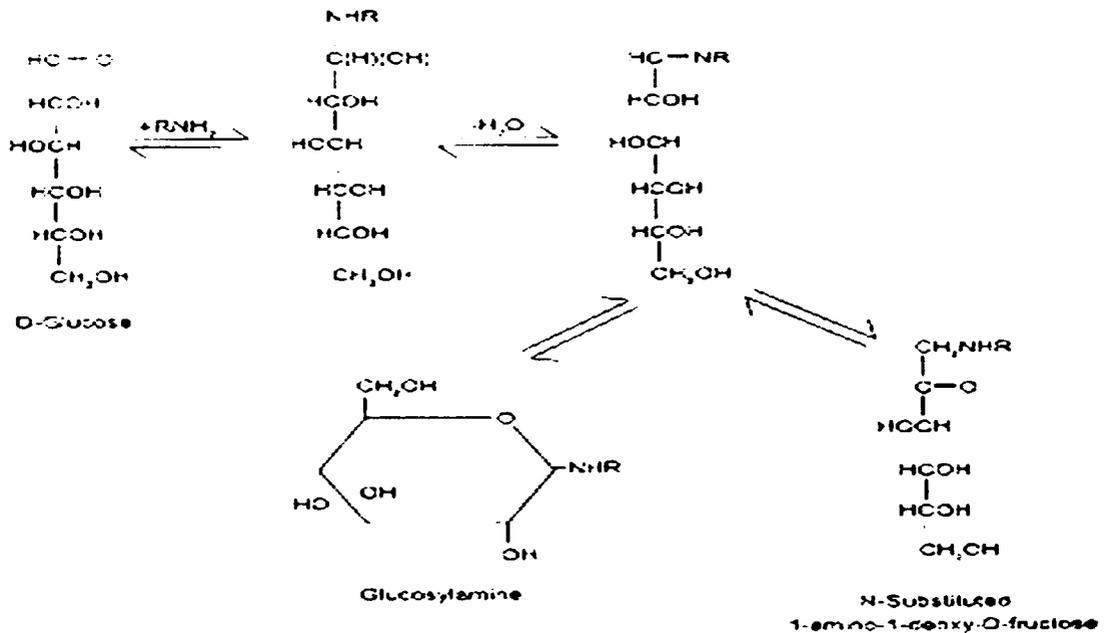
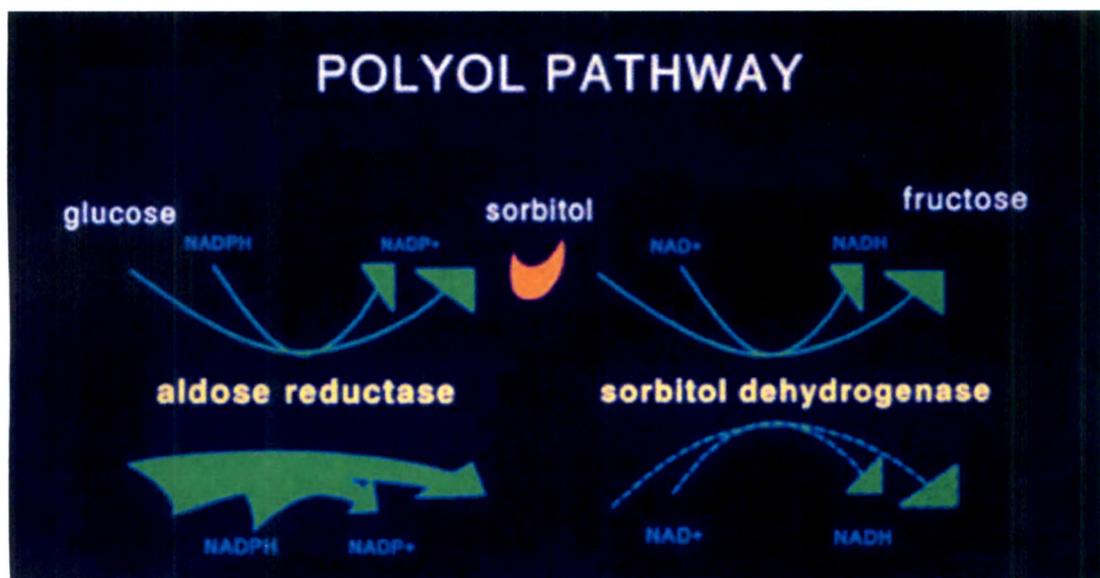


Fig 4: Amadori Rearrangement



Polyol Pathway

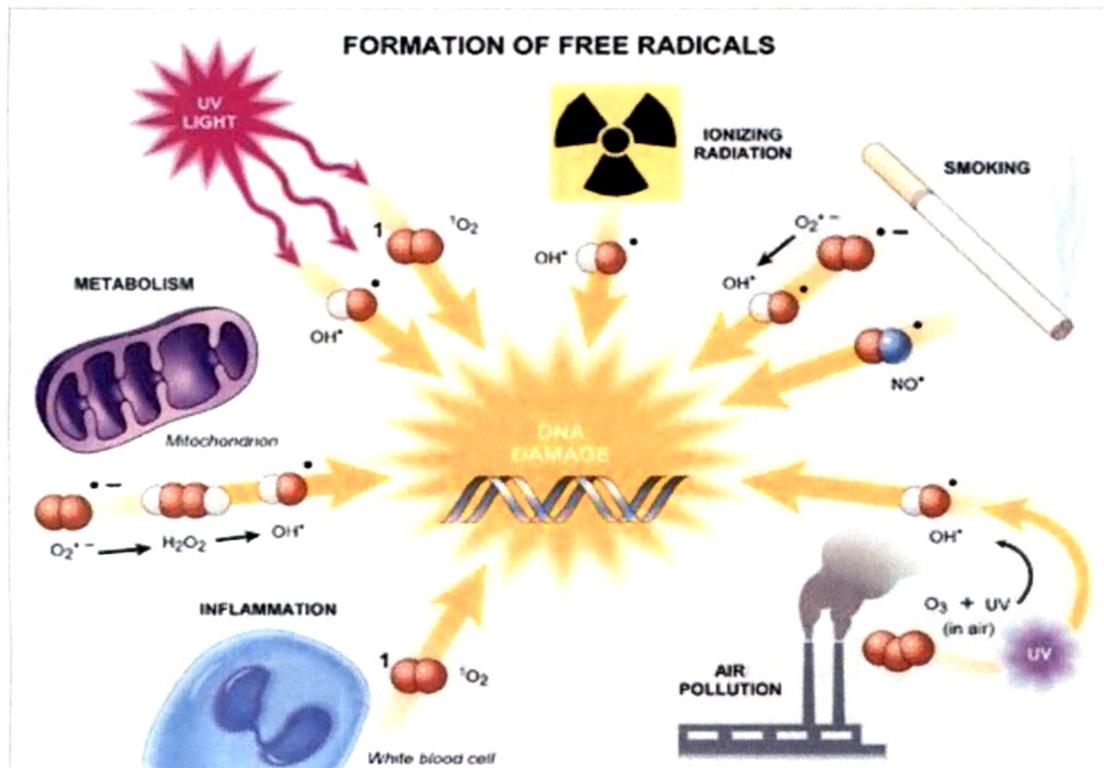
The polyol pathway (sorbitol pathway) has been largely held responsible for diabetic retinopathy. Glucose on reduction by NADPH gives rise to sorbitol, by the action of aldose reductase; the sorbitol so formed is converted to fructose in the presence of sorbitol dehydrogenase (Fig 5). Sorbitol does not diffuse through cell membranes easily and accumulates, causing osmotic stress (Heyningen, 1959). Simultaneously, the myoinositol level falls, which in turn affects the Na⁺K⁺ - ATP ase pump, leading to hydration (Sullis et.al., 1995). But the implications of aldose reductase in development of diabetic retinopathy is a paradox, due to the fact that this enzyme does not function at physiological concentrations as it has a very high Km for glucose, 50 times more than the concentration of 10 – 15 mM in diabetes hydration (Sullis et.al., 1995). The use of sorbinil and other aldose reductase inhibitors has become obsolete now, because experimental evidence has shown this enzyme to be non-functional at levels of physiological concentration of glucose in diabetes



Free radicals

Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases due to various free radicals like – hydroxyl radicals, superoxide radicals, nitric oxide radicals, peroxy radicals. (Beck and Levander, 1998). The exogenous sources of Reactive Oxygen Species (ROS) include electromagnetic radiation, cosmic radiation, cigarette smoke, car exhaust, UV light, ozone, etc (Fig 6). The endogenous sources of ROS are mitochondrial electron transport chain, respiratory burst by phagocytes, beta-oxidation, auto-oxidation, etc. The reduced oxygen products formed during autoxidative glycosylation are superoxide anion (O^{2-}), the hydroxyl radical ($OH\bullet$) and hydrogen peroxide (H_2O_2). All can damage proteins through crosslinking, fragmentation and lipid oxidation (Brownlee et.al., 1988). In tissue not dependent on insulin for glucose uptake (retina, lens, nerve and endothelium), exposure to elevated glucose levels causes an increase in intracellular sorbitol and fructose due to raised aldose-reductase and sorbitol dehydrogenase activity (Hohman and Beg, 1994). The depletion of NADH cell stores by aldose reductase may inhibit the activity of NADH requiring enzymes including nitric oxide (NO) synthase and glutathione (GSH) reductase. Decreased levels of NO can lead to vasoconstriction and tissue injury (Lowenstein et.al., 1994), while reduced levels of GSH increase the susceptibility of endothelial cells to damage by H_2O_2 (Chari et.al., 1984). Since fasting hyperinsulinaemia is considered a hallmark of insulin resistance (DeFronzo and Ferrannini, 1991), a relationship between insulin resistance and plasma free radical concentrations cannot be excluded.

Fig 6: Free Radicals



The genesis of free radical concentrations in insulin resistant conditions might be due to: (1) an insulin-mediated overdrive of sympathetic nervous system activity; (2) a rise in plasma free fatty acid (FFA) concentrations. Enhanced free radical production can move free radical hydrogen atoms to another polyunsaturated fatty acid, thus generating another lipid free radical and lipohydroperoxide (Dianzani, 1990). This latter event produces damage in living cells through at least two mechanisms: (1) mechanical disruption to the membrane causing a loss of the coordinating function of the enzymatic systems contained within it; (2) production of toxic substances from the disintegration of polyunsaturated fatty acids, which are able to migrate from the production sites and reach distant targets.

The production of toxic substances may cause inhibition of a series of enzymatic systems such as microsomal glucose-6-phosphate and plasma membrane Ca/Mg-activated ATPase and Na/K-activated ATPase pump (Dianzani, 1990).

Aminoguanidine, an anti-glycating agent, has been found to be effective in experimentally-induced nephropathy, but leads to adverse hepatocellular and pancreatic toxicity (Sandler, 1993).

Reactive Oxygen Species

The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen ($^3\text{O}_2$). This process is mediated by enzymes such as NAD(P)H oxidases and xanthine oxidase or nonenzymically by redox reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain. In biological tissues superoxide can also be converted nonenzymically into the nonradical species hydrogen peroxide and singlet oxygen ($^1\text{O}_2$) (Steinbeck et al., 1993). In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into the highly reactive hydroxyl radical ($\bullet\text{OH}$). Superoxide and NO are readily converted by enzymes or nonenzymic chemical reactions into reactive nonradical species such as singlet oxygen ($^1\text{O}_2$), hydrogen peroxide, or peroxynitrite (ONOO^-), which can in turn give rise to new radicals.

Reactive Nitrogen Species

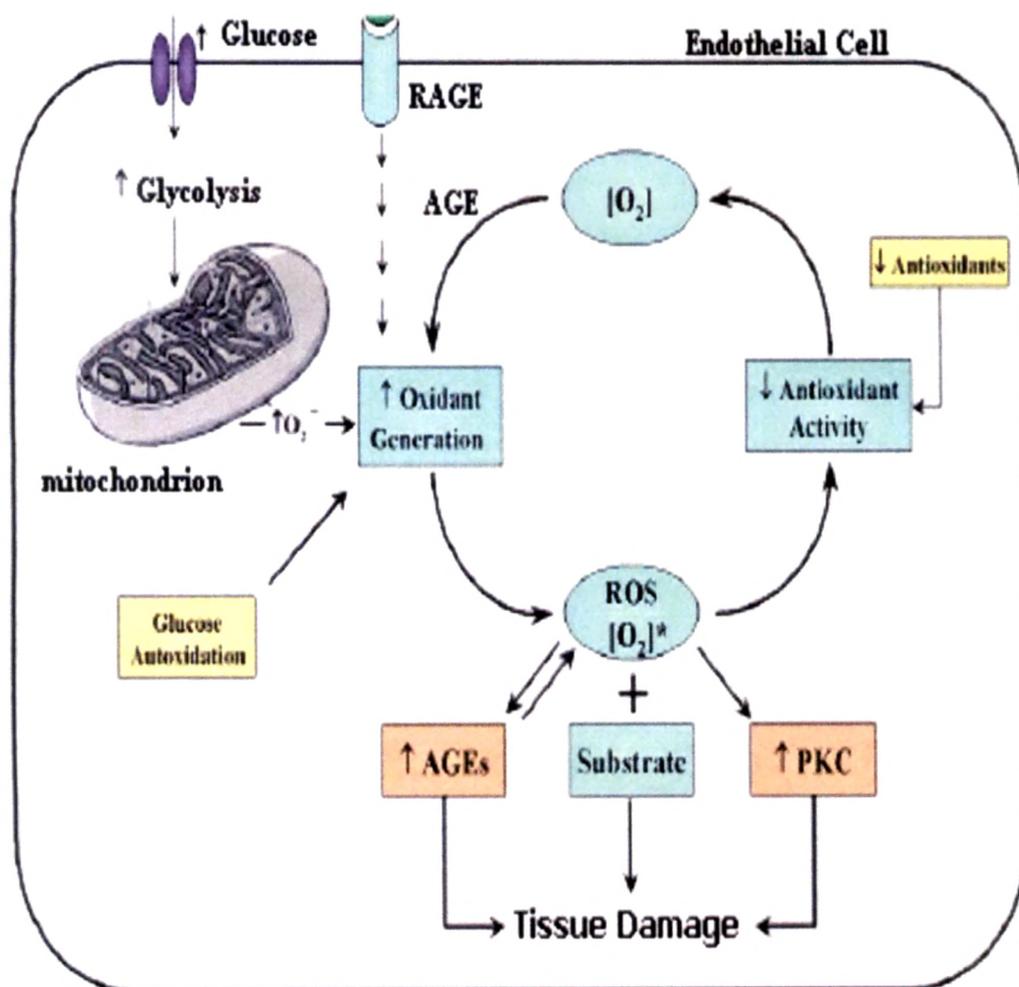
The NO radical ($\text{NO}\bullet$) is produced in higher organisms by the oxidation of one of the terminal guanidonitrogen atoms of L-arginine. This process is catalyzed by the enzyme NOS. Depending on the microenvironment, NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO^+), nitroxyl anion

(NO⁻) or peroxynitrite (ONOO⁻). Some of the physiological effects may be mediated through the intermediate formation of S-nitroso-cysteine or S-nitroso-glutathione (Walker et.al., 2001).

Antioxidant Defense System

To counter the harmful effects of the same, antioxidant defense mechanism operates to detoxify or scavenge these free radicals (Fig 7).

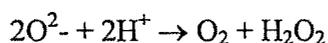
Fig 7: Relationship between rates of oxidant generation, antioxidant activity, oxidative stress, and oxidative damage in diabetes



This defense line consists of antioxidant enzymes like – superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and non-enzymatic molecules like – reduced glutathione (GSH), vitamin C, vitamin E etc. Oxidative stress has been implicated in the pathogenesis of diabetes mellitus. Researchers have found that oxidative stress plays a role in the damage to tissues caused by diabetes due to hyperglycemia. Reports had shown elevated activities in tissue CuZn – SOD, glutathione reductase, catalase and decreased levels of reduced glutathione (Wohaieb and Godin, 1987) along with increased lipid peroxidation (Asayama et.al., 1989) in experimentally induced diabetic animals. Similarly, onset of diabetes altered the antioxidant status and defense system of diabetic patients also (Dominguez et.al., 1998; Telci et.al., 2000; Sekeroglu et.al., 2000).

Superoxide dismutase (SOD) (EC 1.15.1.1)

The enzyme superoxide dismutase, or SOD, is a metalloenzyme which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

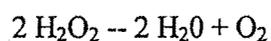


Three isozymes of superoxide dismutase identified in mammals are mitochondrial MnSOD, which contains a manganese ion, CuZn containing extracellular SOD (EC SOD) and CuZnSOD, found primarily in the cytoplasm and nucleus of cells. The Cu-Zn enzyme is a dimer of molecular weight 32,500. The two subunits are joined by a disulfide bond (Keele et.al., 1971). The CuZn SOD gene, SOD1, is located on human Chromosome 21 at location 21q22.1. An alanine (GCT) to valine (GTT) substitution at position -9 in the signal peptide of human Mn-SOD has been shown to change the structural conformation of the mitochondrial targeting sequence of the enzyme leading to

misdirected intracellular trafficking (Shimoda-Matsubayashi, 1996). Extracellular superoxide dismutase (EC-SOD) has an amino acid substitution Arg213Gly in the heparin-binding domain (Sandstrom et.al., 1994). The glycine variant of the enzyme is responsible for high EC-SOD levels in serum (Yamada et.al., 1997) that are correlated with a decrease in nitric oxide production in epithelial cells (Adachi and Wang, 1998) and various other metabolic cardiovascular risk factors (Marklund et.al., 1997).

Catalase (CAT) (EC 1.11.1.6)

Catalase (EC 1.11.1.6), present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The mechanism of catalysis is not fully elucidated, but the overall reaction is as follows:

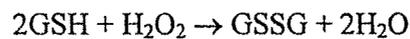


The protein exists as a dumbbell-shaped tetramer of four identical subunits (220,000 to 350,000 kD). Each monomer contains a heme prosthetic group the catalytic center. Catalase monomers from certain species (e.g. cow) also contain one tightly bound NADP per subunit. This NADP may serve to protect the enzyme from oxidation by its H_2O_2 substrate (Eventoff, 1976). The CAT gene is located on human chromosome 11 at location 11p13. There are reports on the quantitative deficiency of catalase leading to cumulative oxidant damage of pancreatic b-cells and were at risk for atherosclerosis and diabetes (Goth and Eaton, 2000). The kind of diabetes involved with catalase deficiency may not be classic type 2 diabetes in which individuals progress from impaired glucose tolerance with modest hyperglycemia and hyperinsulinemia to overt diabetes. The low insulin and C-peptide values in most of the nondiabetic hypocatalasemic subjects and in

the diabetic subjects with catalase deficiency indicate that most diabetic and diabetes susceptible individuals with catalase deficiency may not have hyperinsulinemia of classic type 2 diabetes.

Glutathione Peroxidase (GPx) (EC 1.11.1.9)

The GPx gene is located on human chromosome 3 at location 3p21.3. It has a molecular weight of 84,000 and 4 subunits per mol of enzyme. Glutathione peroxidase catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione as hydrogen donor. Four distinct species of glutathione peroxidase have been identified in mammals to date, the classical cellular enzyme, the phospholipid hydroperoxide metabolizing enzyme, the gastrointestinal tract enzyme and the extracellular plasma enzyme.



Where GSH represents reduced monomeric glutathione, and GSSG represents oxidized glutathione. Glutathione reductase then reduces the oxidized glutathione to complete the cycle:



It has been suggested that this enzyme functions in more times as a mechanism of protecting the cellular membrane system against peroxidative damage. And the importance of selenium as an essential trace element is further concerned with this suggested function of the enzyme. The enzyme is useful for enzymatic determination of lipid hydroperoxide.

Reduced Glutathione (GSH)

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system. Glutathione often attains millimolar levels inside cells, which makes it one of the most highly concentrated intracellular antioxidants. Glutathione exists in two forms: The antioxidant "reduced glutathione" tripeptide is conventionally called glutathione (GSH) and the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide (GSSG). The GSSG/GSH ratio may be a sensitive indicator of oxidative stress (Parris, 1997). Glutathione status is homeostatically controlled both inside the cell and outside, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), its recycling from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

The GSH can act as free-radical scavenger and as an antioxidant enzyme cofactor. Glutathione is most concentrated in the liver (10 mM), where the "P450 Phase II" enzymes require it to convert fat-soluble substances into water-soluble GSH conjugates, in order to facilitate their excretion. The liver parenchymal cells also export GSH to the outside, where it serves as systemic source of -SH/reducing power. GSH depletion leads to cell death, and has been documented in many degenerative conditions. Mitochondrial GSH depletion may be the ultimate factor determining vulnerability to oxidant attack.

Glutathione is an essential cofactor for antioxidant enzymes, namely the GSH peroxidases (both Se-dependent and non-Se-dependent forms exist) and the more recently

described phospholipid hydroperoxide GSH peroxidases. The GSH peroxidases serve to detoxify peroxides (hydrogen peroxide, other peroxides) in the water-phase, by reacting them with GSH; the latter enzymes use GSH to detoxify peroxides generated in the cell membranes and other lipophilic cell phases. Enzymes collectively known as GSH transhydrogenases use GSH as a cofactor to reconvert dehydroascorbate to ascorbate, ribo-nucleotides to deoxyribonucleotides, and for a variety of -S-S- \leftrightarrow -SH inter-conversions.

Microangiopathy

Increases in blood glucose concentrations increase the intracellular accumulation of both glucose and its subsequent metabolic products leading to oxidative stress as discussed earlier. Excess oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. Accumulation of lipids and lipoproteins in the vessel wall, resulting in fatty streaks, is a diabetic microangiopathy is characterized by the progressive damage of endothelium and associated mural cells in microvascular beds, resulting in capillary occlusion, ischemia, and organ failure. These changes occur in the kidney, retina, and microvasculature of peripheral nerves (Vinik et.al., 1975). The short-term effect of high glucose concentration depends on a combined oxidative and nitrosative stress with peroxynitrite formation, whereas the long-term effect is related to ROS generation; in both cases, PKC ultimately mediates permeability changes (Pricci et.al., 2003). Increased production of reactive oxygen species and loss of endothelial NO bioactivity are key features of vascular disease states such as diabetes mellitus (Harrison, 1998). NO is generated in the vascular wall by eNOS, which oxidizes L-arginine to L-citrulline using molecular oxygen. Although

endothelial NO bioactivity is decreased in diabetes, levels of eNOS mRNA and protein are maintained or even enhanced but are associated with reduced NO production and increased superoxide production due to enzymatic "uncoupling" of eNOS (Cosentino et.al., 1997) Tetrahydrobiopterin (BH4) is a required cofactor for eNOS activity (Cosentino and Luscher, 1999); pharmacologic studies suggest that BH4 may mediate some of the adverse effects of diabetes on eNOS function. The exact role of BH4 in NOS catalysis remains incompletely defined, but it appears to facilitate electron transfer from the eNOS reductase domain and maintains the heme prosthetic group in its redox active form (Stuehr, 1999), promotes formation of active NOS homodimers (Tzeng et.al., 1995) and reduces superoxide production by "uncoupled" eNOS (Vasquez-Vivar et.al., 1998). Intracellular BH4 levels are regulated by the activity of the de novo biosynthetic pathway. Guanosine triphosphate cyclohydrolase I (GTPCH, EC3.5.4.16) catalyzes GTP to dihydroneopterin triphosphate. BH4 is generated by further steps catalyzed by 6-pyruvoyltetrahydropterin synthase (PTPS) and sepiapterin reductase (Thony et.al., 2000). GTPCH appears to be the rate-limiting enzyme in BH4 biosynthesis; overexpression of GTPCH is sufficient to augment BH4 levels in cultured endothelial cells (Cai et.al., 2002). Recent findings also suggest that eNOS regulation is abnormal in diabetes, resulting in a direct contribution by eNOS to vascular superoxide production, and that this eNOS dysfunction is related to reduced BH4 availability (Guzik, et.al., 2002). Studies confirm that loss of BH4 in diabetes is the consequence of increased vascular oxidative stress rather than decreased biosynthesis of biopterins (Alp et.al., 2003)

The deleterious actions of diabetes and stress may also increase oxidative stress in the brain, leading to increases in neuronal vulnerability. Increases in oxidative stress may

contribute to stress- and diabetes-mediated decreases in hippocampal neuronal glucose utilization. Neuron specific glucose transporter, GLUT3, immunoprecipitated from hippocampal membranes of diabetic rats subjected to stress exhibited significant increases in HNE immunolabeling compared to control rats, suggesting that HNE protein conjugation of GLUT3 contributes to decreases in neuronal glucose utilization observed during diabetes and exposure to stress. Collectively, these results demonstrate that the hippocampus is vulnerable to increases in oxidative stress produced by diabetes and stress. In addition, increases in HNE protein conjugation of GLUT3 provide a potential mechanism for stress- and diabetes-mediated decreases in hippocampal neuronal glucose utilization (Reagan et.al, 2000).

Advanced Glycated End products (AGEs) are elevated in tissues of diabetic patients with end-stage renal disease, and serum AGE levels correlate with serum creatinine (Makita et.al., 1991). In a recent study on kidney biopsies of patients with type 2 diabetes mellitus and overt nephropathy, it was seen that angiotensin-converting enzyme (ACE) immunostaining was elevated in tubular cells and appeared in interstitial cells; with downregulation of AT1 and upregulation of AT2 receptors. An activation of NF Kappa B and dependent chemokines and Ang II were correlated with proteinuria and interstitial cell infiltration (Mezzano et.al., 2003). Control of hypertension is more important as it appears to be the deleterious factor in the progression of diabetic nephropathy than hyperglycemia. A low protein diet may reduce diabetic hyperfiltration (Pedersen et.al., 1988), diminish proteinuria (Cohen et.al., 1987), and slow the rate of disease progression (Evanoff et.al., 1987). Collagen-collagen cross linking by the oxidation of SH groups as a result of protein glycation, may be particularly responsible

for the basement membrane thickening which is considered to be one of the common histopathological changes observed in diabetic retinopathy, neuropathy and nephropathy (Brownlee et.al., 1988). Increased accumulation of advanced glycated end products (AGEs) has been reported in epiretinal membranes surgically excised from patients with diabetic retinopathy, using immunohistochemical techniques (Bucla et.al., 1994). Retinal microcapillaries are lined by endothelial cells and pericytes. Pericyte controls the integrity of the endothelium, endothelial cell proliferation and maintenance of blood retinal barrier by maintaining endothelial cell/pericyte ratio as 1:1. Loss of pericytes has been reported to be the hallmark of diabetic retinopathy (Kuwabara and Kogan, 1960). The capillary pericytes contain abundant actin fibrils, which like other proteins are prone to glycation. AGE binding to its receptors (RAGE) in pericytes exerts selective toxicity resulting in their death (Yamagishi et.al., 1997).

Macroangiopathy

Endothelial cells play an important role in arterial relaxation because they produce NO, a potent endogenous nitrovasodilator that modulates vascular tone by increasing the production of cGMP (Vallance et.al., 1989). Development of atherosclerosis based on metabolic disorders is accompanied by a smoothed response of morphologically unaltered blood vessels to endothelium-dependent vasodilators such as acetylcholine and bradykinin (Cohen, 1993). This decreased vasodilatory response that precedes overt atherosclerosis is called endothelial dysfunction (Harrison, 1993). Although individual vascular beds may be affected differently by diabetes, probably as a function of different NO synthase activity (Lowenstein and Synder, 1992), several of the known properties of NO (inhibition of platelet adhesion and aggregation, reduction of

monocyte adhesion to endothelial cells, inhibition of the proliferation of vascular smooth cells, and abrogation of the ability of monocytes to oxidize LDLs) (Cooke and Tsao, 1993) suggest that if its effects were reduced this could predispose to atherogenesis.

Diabetic Angiopathy

Cardiovascular disease is generally similar in patients with IDDM or NIDDM and patients without diabetes. **Heart disease** accounts for approximately 50% of all deaths among people with diabetes in industrialized countries. Risk factors for heart disease in people with diabetes include smoking, high blood pressure, high serum cholesterol and obesity. Patients with NIDDM (and patients with impaired glucose tolerance) are commonly obese and have hypertension and dyslipidemia (increased serum triglyceride and decreased high-density lipoprotein cholesterol concentrations). Diabetes negates the protection from heart disease which pre-menopausal women without diabetes experience. Recognition and management of these conditions may delay or prevent heart disease in people with diabetes. Coronary artery disease or blockage of the arteries supplying the heart is the major cause of death in patients with diabetes mellitus. It can result in heart attacks, heart failure or angina. The risk of developing coronary artery disease in diabetic patients is known to be several times higher at every level of cholesterol. The multiple risk factor intervention trial (MRFIT) found that coronary artery disease risk in diabetic subjects at any given plasma cholesterol level was approximately four times greater than in non-diabetic patients. This is especially true in women who lose their "natural" protection against heart disease. With respect to heart disease, diabetes mellitus is more than just a problem of high blood sugar. In contrast to eye and kidney disease, good blood sugar control alone is not enough to prevent the development of heart disease. Diabetes

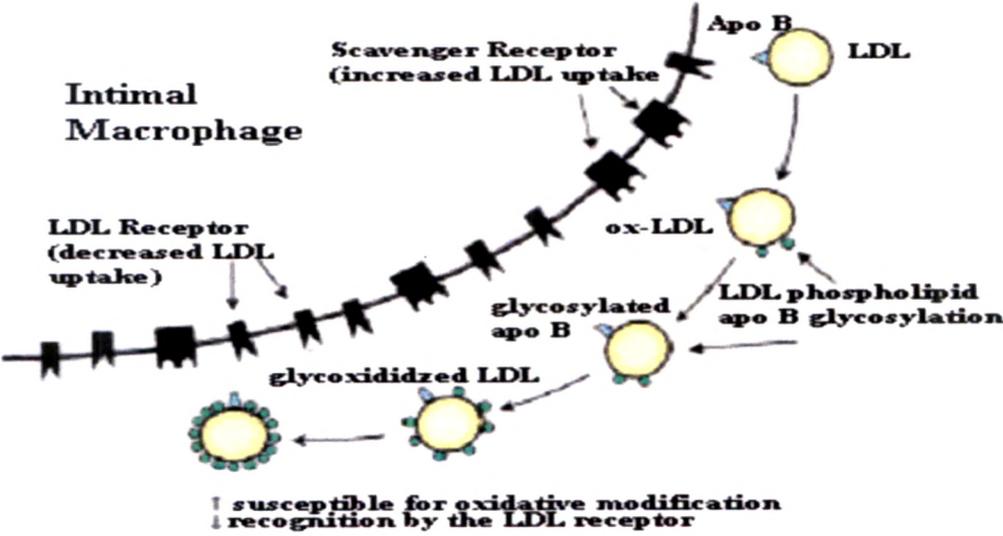
mellitus is associated with widespread abnormalities in the blood. Of particular importance to heart disease are the blood lipids, which includes cholesterol and triglyceride. High triglyceride and low HDL cholesterol (the good cholesterol) is often seen in diabetic patients. In addition, the LDL cholesterol (the bad cholesterol) in diabetics may be 10-15% higher than in non-diabetics.

Atherosclerosis

Patients with diabetes mellitus are particularly susceptible to morbidity and mortality resulting from cardiovascular diseases, especially atherosclerosis. Diabetes and coronary heart disease share many of the same risk factors, such as disorders of lipid metabolism and hypertension. Hyperglycemia induces a large number of alterations at the cellular level of vascular tissue that potentially accelerate the atherosclerotic process. Nonenzymatic glycosylation of proteins and lipids which can interfere with their normal function by disrupting molecular conformation, alter enzymatic activity, reduce degradative capacity, and interfere with receptor recognition. In addition, glycosylated proteins interact with a specific receptor present on all cells relevant to the atherosclerotic process, including monocyte-derived macrophages, endothelial cells, and smooth muscle cells. The interaction of glycosylated proteins with their receptor results in the induction of oxidative stress and proinflammatory responses, oxidative stress, protein kinase C (PKC) activation with subsequent alteration in growth factor expression. Importantly, these mechanisms may be interrelated (Nishikawa et.al., 2000). Transvascular LDL transport may be increased in patients with type 1 diabetes suggesting that lipoprotein influx into the arterial wall is increased, possibly explaining accelerated development of atherosclerosis (Kornerup et.al., 2003). LDL glycosylation is increased in correlation

with glucose levels, and AGE-ApoB levels are up to 4-fold higher in diabetic patients (Bucala et.al., 1993) (Fig 8). The oxidation of low density lipoproteins (LDL) is considered a key event in the initiation of atherosclerosis (Astley et.al., 1999). Although the exact mechanisms responsible for accelerated atherogenesis in patients with diabetes are not completely understood, an important role may be played by increased glycosylation of lipoproteins. The established association between atherosclerosis and lipid peroxidation within the vascular wall has led to a renewed interest in the oxidative stress of hyperglycaemia as a potential mechanism for diabetic vascular disease. LDL glycosylation enhances its uptake by human aortic intimal cells (Sobenin et.al., 1993) and monocyte derived macrophages (Klein et.al., 1995) with stimulation of foam cells formation, the recognition of glycated LDL by the scavenger receptor pathway is thought to promote intracellular accumulation of cholesteryl esters and promote atherosclerosis.

Fig 8: Potential mechanisms by which LDL glycosylation increases its atherogenicity



RAGE is reported to intercept diverse processes such as endothelial leakage and cellular activation can also occur by nonreceptor pathways, or by intracellularly generated glycoxidant derivatives, leading to ROS generation and oxidant stress (Radoff et.al., 1998). Another example is the alterations in normal function of the complement regulatory protein. Glycation of the complement regulatory protein CD59 results in its inactivation (Acosta et.al., 2000) and may increased the sensitivity of the diabetic endothelium to MAC-induced release of growth factors and cytokines. Glycosylation of matrix components such as collagen VI, laminin, and vitronectin decreased binding of anionic heparan sulfate, leading to greater turnover of heparin sulfate (Brownlee et.al., 1988). AGE interaction with RAGE on endothelial cells results in the induction of oxidative stress and consequently of the transcription factor NF- κ B (Yan et.al., 1994) and VCAM-1 (Schmidt et.al., 1995). Pathological studies of human atherosclerotic plaques showed infiltration of RAGE-expressing cells in the expanded intima (Brett et.al., 1993). Monocyte-macrophage interaction with AGEs results also in the production of mediators such as interleukin-1, tumor necrosis factor- α , platelet-derived growth factor, and insulin growth factor-I (Vlassara et.al., 1988), which have a pivotal role in the pathogenesis of atherosclerosis (Ross, 1999) (Fig 9).

High ambient glucose concentrations activate Protein kinase C (PKC) by increasing the formation of diacylglycerol (DAG), the major endogenous cellular co-factor for PKC activation, from glycolytic intermediates such as dihydroxy-acetone phosphate and glyceraldehyde-3-phosphate. collagen and decreasing the synthesis of proteolytic enzymes that degrade matrix proteins (Nabel et.al., 1993). Increased expression of TGF- β is thought to lead to thickening of capillary basement membrane –

one of the early structural abnormalities. The PKC system is ubiquitously distributed in cells and is involved in the transcription of several growth factors, and in signal transduction in response to growth factors (Park et.al., 2000). Oxidative stress may also be involved in the activation of DAG-PKC in vascular tissue (Nishikawa et.al., 2000).

Diabetic Retinopathy

Retinopathy is the commonest complications of diabetes. Surveys show that at any time upto 10 % of people with diabetes will have retinopathy (McLeod et.al., 1988). Approximately 25 % of patients with Type 1 diabetes mellitus have been shown to be affected with retinopathy, with the incidence increasing to 60 % after 5 years and 80 % after 10 – 15 years of affliction. However since there are more adult onset cases than juvenile ones, Type 2 diabetes mellitus accounts for a higher proportion of patients with visual impairment. There are two kinds of diabetic retinopathy. The most common is called non- proliferative diabetic retinopathy (NPDR) or "background" retinopathy. This type affects approximately 90-95% of long term diabetics to some degree. With diabetes, the tiny retinal blood vessels can weaken, leaking fluid or blood into the retina, and failing to provide proper nutrients (necessary to every part of the human body) to the retina. Proliferative diabetic retinopathy (PDR) is a more advanced and severe form of retinopathy. In this type, the retinal vessels close, fostering growth of abnormal blood vessels (neovascularization) over the retina that may even grow into the vitreous.

It is a leading cause of blindness and visual disability. Cataracts and glaucoma are more common in patients with diabetes mellitus. In addition, it can affect the part of the eye at the back which is responsible for sensing light and colour, the retina. In the retina, small vessels become "leaky" resulting in the formation of exudates. If these exudates are

too close to the most sensitive area of the retina, the macula, this can impair your vision. Findings, consistent from study to study, make it possible to suggest that, after 15 years of diabetes, approximately 2% of people become blind, while about 10% develop severe visual handicap. Diabetes mellitus also causes weakness in the blood vessel walls causing them to bulge and form microaneurysms. Generally, all persons with diabetes mellitus develop some of these microaneurysms or exudates if they have diabetes for a sufficient length of time. Loss of vision and blindness in persons with diabetes can be prevented by early detection and treatment of vision-threatening retinopathy: regular eye examinations and timely intervention with laser treatment, or through surgery in cases of advanced retinopathy. Nevertheless, the development of retinopathy is generally duration-dependent in NIDDM, as it is in IDDM (Palmberg, et.al., 1981).

Diabetic Nephropathy

Diabetes is among the leading causes of **kidney failure**, but its frequency varies between populations and is also related to the severity and duration of the disease. Approximately, 30-50% of patients with diabetes mellitus may develop kidney disease. Several measures to slow down the progress of renal damage have been identified. They include control of high blood glucose, control of high blood pressure, intervention with medication in the early stage of kidney damage, and restriction of dietary protein. It begins with the appearance of small amounts of a protein (30 to 300 mg of albumin per 24 hours), called albumin in the urine. This is called microalbuminuria. After another 5 to 10 years of diabetes, overt proteinuria (>500 mg of protein per liter, equivalent to >300 mg of albumin per 24 hours) develops in patients destined to have end-stage renal disease. Hypertension invariably develops during this period. In the next 5 to 10 years, the

nephrotic syndrome develops and the glomerular filtration rate falls, resulting in end-stage renal disease. Initially, there is renal hypertrophy, with expansion of the glomeruli, including the mesangium and glomerular basement membrane, and an increase in kidney size (Feldt-Rasmussen et.al., 1991). Glomerular composition changes more slowly, leading to characteristic mesangial expansion, thickening of the glomerular basement membrane, and afferent and efferent arteriosclerosis (Mauer et.al., 1984). End-stage renal disease is characterized by small, atrophic kidneys with diffuse glomerulosclerosis. The capacity to clear macromolecules is impaired in diabetes (Mauer et.al., 1979). Accumulation of albumin and larger proteins within the glomerular wall and in the mesangium may stimulate mesangial matrix production and lead to the diffuse and nodular changes of diabetic nephropathy.. Five years after the onset of diabetes, hyalinosis of the efferent glomerular arterioles and early glomerular nodules (Kimmelsteil-Wilson nodules) may be detectable (Takazakura et.al., 1975). All patients with Type 1 diabetes progress through an initial clinically silent phase that lasts approximately 10 years. The only detectable changes in this latent phase are mild nephromegaly and an increase in the glomerular filtration rate (GFR) due to increased renal blood flow (hyperfiltration). Progressive renal disease characterized by gross proteinuria, blood pressure elevation and diminished glomerular filtration rate is not helped by intensive insulin therapy. Related-donor renal transplants and transplants between HLA-matched donors are more successful (Jacobson et.al., 1988) by meticulous postoperative control of diabetes in the hope of retarding the development of nephropathy in the transplanted kidney.

Diabetic Neuropathy

It is probably the most common complication of diabetes. Studies suggest that up to 50% of people with diabetes are affected to some degree. Major risk factors of this condition are the level and duration of elevated blood glucose. Neuropathy can lead to sensory loss and damage to the limbs. It is also a major cause of impotence in diabetic men. The three recognized forms of diabetic neuropathy are mononeuropathy involving a peripheral or cranial nerve, symmetrical peripheral polyneuropathy (most common) and autonomic neuropathy (Vinik and Mitchell, 1988). Diabetic mononeuropathy may involve the femoral, obturator, sciatic, median, or ulnar nerve, or it may affect a cranial nerve in isolation; causing wrist drop, foot drop or paralysis of the third, fourth or sixth cranial nerve. Patients with this form of neuropathy usually have had IDDM or NIDDM for at least 20 years. The most common manifestation of the peripheral neuropathy of diabetes is symmetrical sensory loss in the distal lower extremities (Vinik and Mitchell, 1988) with symptoms including numbness, tingling and burning that is worse at night. Diminished sensory perception in diabetic neuropathy may lead to injuries to skin and joints, causing calluses, ulceration, and neuropathic arthropathy (Charcot's Joints).

Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the most costly complications of diabetes, especially in communities with inadequate footwear. It results from both vascular and neurological disease processes. Diabetes is the most common cause of non-traumatic amputation of the lower limb, which may be prevented by regular inspection and good care of the foot.

Diabetic ketoacidosis occurs when there is insufficient insulin to deal with the amount of sugar in the blood stream. When this occurs, the body uses fat as an energy source and this results in the production of ketones that accumulate in the body. These ketones also appear in the urine and can be detected with a simple urine labstix test. Diabetic ketoacidosis often occurs in type 1 diabetes mellitus when the patient does not give him/herself insulin injections. In type 2 diabetes mellitus, it usually occurs when a patient has some other illness at the same time. This would include all types of infections or fever such as urine infection or chest infections. Other types of stressful events can also lead to diabetic ketoacidosis such as a heart attack. The symptoms of diabetic ketoacidosis include thirst, passing large volumes of urine, feeling very tired, nausea, vomiting and abdominal pain. Others may notice very deep, rapid breathing and a fruity smell on the breath. In severe cases, patients can become drowsy and become unconscious.

Compared to diabetic ketoacidosis, which can occur very quickly, **hyperosmolar non ketotic coma** occurs more gradually. The patient may feel thirsty and pass large volumes of urine. This will result in your becoming more and more dehydrated. You will feel tired and may lose weight. Usually the urine ketones are negative or present only in small quantities. Patients may become more and more drowsy and become unconscious. This condition is more common in type 2 diabetes mellitus and may occur because of insufficient medication.

Dyslipidemia

Lipids

Lipids are substances of biological origin that are soluble in organic solvents such as chloroform and methanol but are only sparingly soluble, if at all, in water. Dietary lipids (fats) consist mainly of heterogeneous mixtures of triglycerides and small proportion of phospholipids. They also contain about 1 – 3 % of other fat soluble chemicals collectively designated as non-glyceride fraction (NGF) which includes cholesterol, other sterols, fat soluble vitamins (carotenes, Vit A, D and E), lignans, etc. (Ghafoorunnisa, 1998).

Triacylglycerols function as energy reservoirs in animals and are therefore the most abundant class of lipids even though they are not components of biological membranes. Fats and oils (which differ only in that fats are solid in room temperature and oils are liquid) are complex mixtures of simple and mixed triacylglycerols with varied fatty acid composition.

Cholesterol is a major component of animal plasma membranes and occurs in lesser amounts in the membranes of their subcellular organelles. Its polar OH group gives it a weak amphiphilic character, whereas its fused ring system provides it with greater rigidity than other membrane lipids. It is also abundant in lipoproteins, where ~ 70% of it is esterified to long-chain fatty acids to form cholesteryl esters. Cholesterol is the metabolic precursor of steroid hormones, substances that regulate a great variety of physiological functions including sexual development and carbohydrate metabolism. Plants contain little cholesterol. Rather, the most common sterol components of their

membranes are stigmasterol and β -sitosterol which differ from cholesterol only in their aliphatic side chains.

Digestion and absorption

A lingual lipase is secreted by Ebner's glands on the dorsal surface of tongue, and the stomach also secretes a lipase. The gastric lipase is of little importance except in pancreatic insufficiency but lingual lipase is active in stomach and can digest as much as 30% of dietary triglycerides.

Most fat digestion begins in the duodenum. Fats are finely emulsified in the small intestine by the detergent action of bile salts, lecithin and monoglycerides. Lipids and bile salts interact and form micelles. Colipase, which is secreted in pancreatic juice is activated in the intestinal lumen by trypsin and this facilitates exposure of active site of pancreatic lipase which splits fats into free fatty acids and two monoglycerides. It acts on fats that have been emulsified. Bile salt activated lipase catalyzes the hydrolysis of cholesterol ester, esters of fat-soluble vitamins, phospholipids and triglycerides. Hydrolase enzymes in the intestinal lumen hydrolyze this cholesterol ester and cholesteryl ester.

The lipids were thought to enter the enterocytes by passive diffusion, lipids are rapidly esterified inside the cells. Fatty acids containing more than 10-12 carbon atoms are reesterified to triglycerides in the mucosal cells. In addition, some of the absorbed cholesterol is also esterified. Closely related sterols of plant origin are poorly absorbed. Non-absorbable plant sterols such as those found in soybeans reduce the absorption of cholesterol, for esterification with fatty acids. The triglycerides and cholesteryl esters are then coated with a layer of protein, cholesterol and phospholipid to form chylomicrons in

the enterocyte and then enter the circulation via the lymphatic. After the chylomicrons discharge their triglycerides, the cholesterol is transported to the liver.

Cholesterol is used for the manufacture and repair of cell membranes, for synthesis of bile acids and vitamin D, and is the precursor of five major classes of steroid hormones: progestins, glucocorticoids, mineralocorticoids, androgens and estrogens. Liver is the major site of cholesterol synthesis. Extra-hepatic tissues also synthesize cholesterol. Cholesterol is formed from acetyl-CoA. Three molecules of acetyl-CoA are condensed to produce 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA), which, in turn, is converted to mevalonic acid through the action of HMG-CoA reductase. Mevalonic acid is converted into squalene after a series of condensation and rearrangement steps. Squalene then cyclizes to form lanosterol, which is further modified to yield cholesterol.

HMG-CoA reductase regulate the synthesis of cholesterol in the liver, which is the rate limiting enzyme in cholesterol biosynthesis. In the liver, some of the cholesterol is degraded to bile acids, primarily cholic acid and chenodeoxycholic acid and also excreted out through faeces as neutral sterols.

Lipoproteins

Lipids, such as, phospholipids, triacylglycerols, and cholesterol, are transported by the circulation as components of lipoproteins, globular micelle like particles that consist of a nonpolar core of triacylglycerols and cholesteryl esters surrounded by an amphiphilic coating of protein, phospholipid, and cholesterol (Kane, 1991). The lipoproteins are classified into five categories (Table II). Lipoproteins transport lipids in three separate but interacting pathways through the body – the exogenous, endogenous

and reverse cholesterol transport pathways. The exogenous pathway transports dietary lipids, through the formation of chylomicrons in the small intestine, to the liver; the endogenous pathway is responsible for transporting hepatic lipids by way of VLDL and LDL to the peripheral tissues; and the reverse cholesterol transport pathway uses HDL to transport cholesterol from the peripheral tissues back to the liver for excretion or reuse. The clearance of chylomicrons are rapidly carried out by the enzyme, lipoprotein lipase and its action results in the loss of 90% of triglycerides and degrades VLDL into IDL and LDL. The resultant free fatty acids are taken up by extrahepatic tissues. Fibroblasts, lymphocytes, arterial smooth muscle cells and liver have special receptors for LDL and 50% of them are degraded in the liver and 50% in extrahepatic tissues. Nascent HDL produced in the liver and intestine absorb free cholesterol from peripheral cells and convert it to cholesteryl ester for storage inside the HDL core during transport. Both lecithin-cholesterol acyltransferase (LCAT) and apolipoproteins A-I and D are essential for the esterification of cholesterol. Through chylomicron remnants and LDL esterified cholesterol is taken up by liver and converted to bile salts.

Apolipoproteins

The protein components of lipoproteins are known as apolipoproteins or just apoproteins. They are very important since they provide structural stability to the lipoproteins, and a number of apoproteins function as ligands in lipoprotein-receptor interactions or are cofactors in enzymatic processes that regulate lipoprotein metabolism. Most of them are water soluble and associate rather weakly with lipids. Hence they readily transfer between lipoprotein particles via the aqueous phase (Table III).

Table II : Characteristics of Plasma Lipoproteins

Lipoprotein Class	Density of flotation, g/ml	Major Lipid Constituent	TG/CHOL ratio	Significant apoproteins	Site of synthesis	Mechanism(s) of catabolism
Chylomicrons and remnants	<< 1.006	Dietary triglycerides and cholesterol	10:1	A-I, A-IV, B-48, E, C-I, C-II, C-III	Intestine	Triglyceride hydrolysis by lipoprotein lipase ApoE-mediated remnant uptake by liver.
VLDL	< 1.006	Endogenous or hepatic triglycerides	5:1	B-100, E, C-I, C-II, C-III	Liver	Triglyceride hydrolysis by lipoprotein lipase
IDL	1.006 – 1.019	Cholesteryl esters and endogenous triglycerides	1:1	B-100, E, C-II, C-III	Catabolic product of VLDL	50% converted to LDL mediated by hepatic lipase. 50% apo-E mediated uptake by liver.
LDL	1.019 – 1.063	Cholesteryl esters	NS	B-100	Catabolic product of VLDL	ApoB-100 mediated uptake by LDL receptor (~75% in liver)
HDL	1.063 – 1.21	Phospholipids, cholesteryl esters	NS	A-I, A-II, C-I, C-II, C-III, E	Intestine, liver, plasma	Transfer of cholesteryl ester to VLDL and LDL Uptake of HDL cholesterol by hepatocytes.
Lp(a)	1.05 – 1.09	Cholesteryl esters	NS	B-100, apo(a)	Liver	Unknown

Goodman and Gilman's: The pharmacological basis of therapeutics. Editors: Hardman JG, Limbird LE, Ed 10th, 2001, McGraw-Hill, medical publishing division, USA.

Table III : Apolipoproteins and their function

Apolipoprotein	Average concentration, mg/dl	Sites of synthesis	Functions
Apo A-I	130	Liver, intestine	Structural in HDL; LCAT cofactor; ligand for HDL receptor; reverse cholesterol transport
Apo A-II	40	Liver	Forms -S-S- complex with apoE-2 and E-3, which inhibits E-2 and E-3 binding to lipoprotein receptors.
Apo B-100	85	Liver	Structural protein of VLDL, IDL, LDL; LDL receptor ligand
Apo B-48	Fluctuates according to dietary intake.	Intestine	Structural protein of chylomicrons
Apo C-I	6	Liver	LCAT activator. Modulates receptor binding of remnants.
Apo C-II	3	Liver	Lipoprotein lipase (LPL) cofactor.
Apo C-III	12	Liver	Modulates receptor binding of remnants.
Apo E	5	Liver, brain, skin, gonads, spleen	Ligand for LDL receptor and receptors binding remnants; reverse cholesterol transport (HDL with apoE).
Apo (a)	Variable (under genetic control)	Liver	Modulator of fibrinolysis.

LCAT , lecithin cholesterol acyl transferase

Important enzymes involved in lipid and lipoprotein metabolism

- 1) **Lipoprotein lipase (LPL):** synthesized by adipocytes, monocytes, macrophages. Transported to surface of capillary endothelial cells of these tissues and interacts with chylomicron and VLDL mediating the hydrolysis of triglycerides leading to the release of free fatty acid. ApoC-II is an important co-factor for LPL.
- 2) **Hepatic lipase:** synthesized by hepatocytes. Transported to capillary endothelium of adrenals, ovaries and testis, where it functions in the release of lipids from lipoproteins for use in their organs. Its activity is increased by androgens and reduced by estrogens. Apo E may facilitate both triglycerides and phospholipid hydrolysis by hepatic lipase and may be the co-factor for the enzyme.
- 3) **Lecithin : cholesterol acyl transferase (LCAT) :** circulates in association with HDL in the plasma and functions to esterify free cholesterol. The major substrate for LCAT is HDL particle and to a lesser extent, LDL.
- 4) **Cholesterol Ester transfer protein (CETP) :** functions in the transfer of cholesteryl esters from large HDL to VLDL, IDL and remnant lipoproteins. In return, Triglyceride from these lipoproteins is transferred to HDL.

Causes of Dyslipidemia

Dyslipidemia may be caused by a wide range of clinical manifestations which can be classified into (I) Primary and (II) Secondary (Fredrickson and Lees, 1966).

Primary Disorders

Type	Class	Possible reason	Symptoms
Hyperchylomicronemia	I	a) LPL deficiency (homozygous) b) ApoC -II deficiency	Eruptive xanthoma

			c) condition leading to LPL inhibition.	
Hyperlowdensity lipoproteinemia	II	a) Familial defective apo B-100	Coronary artery disease Glomerular disease Fibrositis – arthritis involving particularly ankles and Achilles tendon.	
Hyperlowdensity lipoproteinemia and very low density lipoproteinemia (Broad Beta disease)	III	a) Apo E deficiency	Cataract Retinal degeneration Corneal opacity Glomerular disease	
Hyperverylowdensity lipoproteinemia	IV	a) Familial dyslipidemic hypertension. b) LPL deficiency (heterozygous) c) Hyperapo B-100	Coronary artery disease	
Hyperchylomicronemia	V	a) same as that of Type I but occurs in adulthood.	Eruptive xanthoma Lipemia retinalis Abdominal pain	

Secondary Disorders

Secondary disorder

Diabetes mellitus

Types

Mainly IV and also I, III, V

Alcoholism

Type IV, V

Nephrotic syndrome

II, IV, V

Obstructive jaundice

II

Hypothyroidism

III

Dyslipidemia and atherosclerosis

Hyperlipidaemia has been implicated in atherosclerosis, which is the leading cause of death among world population. In the U.S. 50 % of all deaths with 60 % of death in older people (>60 yrs) occurs due to atherosclerosis and further leads to coronary artery disease (CAD), myocardial infarction, stroke, etc.

CAD rates in urban areas in India are now 4-fold higher than in the United States (US) although the rates were similar in 1968 (Enas, 2000). The prevalence of CAD in urban India (10%) is about double that of rural India (5%) and about 4-fold higher than in the US (2.5%) (Reddy, 1998). The rates appear to be higher in South India, with a major tertiary care centre in Chennai reporting an 8-fold increase in the proportion of CAD patients from 4% to 33% over the past 30 years (Enas, 1998) and in Kerala, Trivandrum having a prevalence of 7% in rural and 13% in urban areas (Enas, 1998). The high rates of CAD in urban India compared to rural India, despite lower rates of smoking, suggest important roles for nutritional and environmental factors. There is a significant increase in body mass index (BMI) in urban India compared to rural India (BMI, 24 versus 20 in men, 25 versus 20 in women). There is also a higher rate of abdominal obesity among the urban population, with urban men having a waist to hip ratio (WHR) of 0.99 compared to 0.95 among rural men. These increases in BMI and WHR result in significant insulin resistance and dyslipidaemia (Enas et. al., 1997). The cholesterol levels are at least 25 mg/dl higher in urban than in rural areas of India. Though contemporary mortality data from India are unavailable, the totality of the data suggests that an epidemic of CAD is already underway.

The excess burden of CAD in Indians is due to a combination of nature (genetic predisposition) and nurture (environmental or lifestyle factors) (Enas et. al., 1997). Decreased physical activity and increased consumption of calories and saturated fat result in abdominal obesity, insulin resistance, and atherogenic dyslipidaemia such as, low levels of HDL, high levels of LDL resulting in a high LDL/HDL ratio, along with abnormal concentrations of other lipoproteins. A diet high in saturated fat includes not only meat but also whole milk as well as high-fat dairy products and certain vegetable oils (coconut, palm and, palm kernel oil). Trans-fatty acids formed during hydrogenation of vegetable oils can raise cholesterol levels (Pekkanen et. al., 1990). The risk of a first Myocardial Infarction (MI) can be reduced by about a third by aggressive lowering of LDL to 110 mg/dl (Brown et. al., 1993). One of the most fascinating developments of the past ten years is the increasing evidence that the inexorable progress of atherosclerosis can be slowed, arrested, and even reversed by aggressive lipid-lowering therapy (Thompson, 1997).

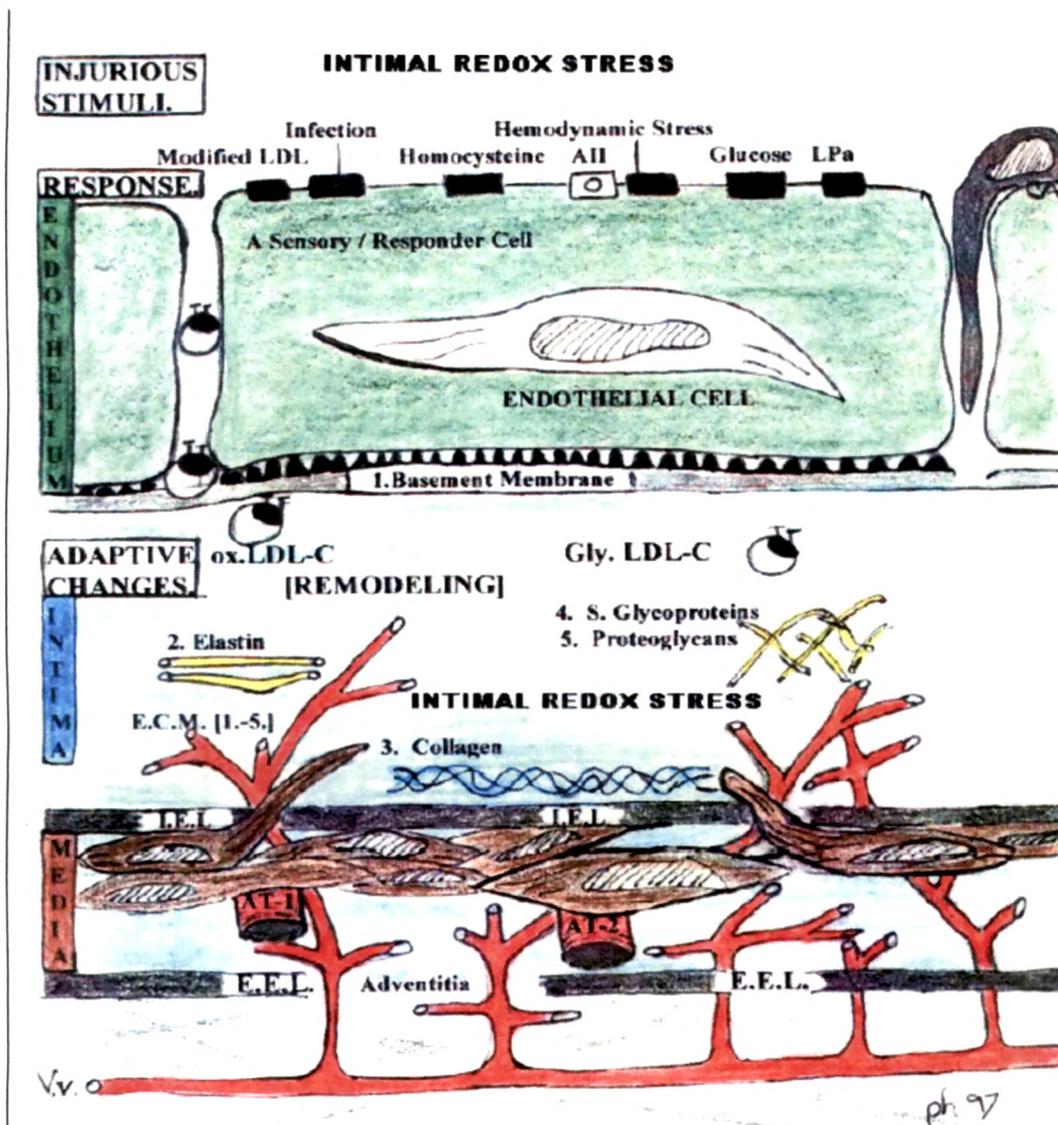
Events of atherosclerosis are multifactorial process. Oxidation and response to injury proposes the major theories leading to the pathogenesis of atherosclerosis. 1. Injury to the inner wall of arteries by lipoprotein derived oxidants. 2. production of cellular adhesion molecules and cytokines for inflammatory cells. 3. Rolling of leukocytes on the monolayer of endothelial cells mediated by selectins. 4. Extravasations of leukocytes into endothelium involving monocyte Chemo attractant protein – 1. 5. Oxidation of lipoprotein by lipoxygenases. 6. Uptake of oxidative LDL and induction of proinflammatory signals i.e. cytokinins like macrophage colony stimulating factor (MCSF) or granulocyte macrophage colony stimulating factors (GM – CSF), to

macrophages. 7. Foam cell formation and foam cell death facilitated by metalloproteinases leading to thrombus formation (Fig 9).

Atherosclerosis is the thickening of the intimal layer of the arterial wall. The normal arterial wall has 3 layers – inner intima, middle media, and outer adventitia. Atherosclerosis develops with the uptake of excess of oxidized LDL (modified LDL) by macrophages in an unregulated manner through receptors unrelated to the LDL receptor (Brown MS & Goldstein JL, 1983) commonly referred to as scavenger receptors. Proliferation of smooth muscle cells and migration of macrophages, which becomes foam cells leads to the formation of fatty streaks which ultimately forms atherosclerotic plaques, which is characterized, by calcification and cell necrosis in that area (Fig 9).

Atherosclerosis causes (1) Occluding the arteries slowly over time (2) Occluding the arteries suddenly by rupture of plaques (3) weakening of walls of arteries (4) Intimal thickening (5) Reduction elasticity of elastic tissue (6) Changes in lipoprotein composition . All these lead to ischemic disease of the heart and ultimately results in coronary artery disease, myocardial infarction, stroke, etc.

Fig 9: Atherosclerosis and oxidative stress



Ref: Hayden MR, Tyagi SC. Intimal Redox Stress: Accelerated atherosclerosis in metabolic syndrome and type 2 diabetes mellitus. *Atherosclerosis*. *Cardiovascular Diabetology* 2002, 1: 3 – 80.

Dyslipidemia and diabetes

Abnormalities in lipoproteins are very common in both individuals with non-insulin-dependent diabetes (NIDDM) and insulin-dependent diabetes (IDDM). They are mainly induced by diabetes associated complications such as obesity and renal disease. In IDDM patients due to the obligatory requirement for insulin therapy, a spectra of situation is possible with greatly elevated glucose, FFA, ketones, lipolytic hormones. Extreme elevations in VLDL levels have been recognized as being a common occurrence in diabetic ketoacidosis (Bagdade et.al., 1967). Elevation in VLDL triglycerides in IDDM are often correlated with the degree of diabetic control (Lopes-Virella et.al., 1981).

In poorly controlled diabetes, triglycerides are increased, LDL cholesterol is increased and HDL cholesterol is decreased. The hypertriglyceridaemia particularly improves rapidly with insulin therapy and an improvement in glycaemic control. Type 1 patients with nephropathy develop protein abnormalities, resulting in a high LDL cholesterol even at the stage of microalbuminuria. Lipid abnormalities occur more frequently in Type 2 diabetes than in the non-diabetic state. The characteristic dyslipidaemia is hypertriglyceridaemia, low HDL cholesterol and high LDL cholesterol. The dyslipidaemia of Type 2 diabetes is closely related to insulin resistance and hyperinsulinaemia and is a component of Syndrome X or Reaven's Syndrome (glucose intolerance, hypertension, dyslipidaemia with accelerated atherosclerosis and truncal obesity).

It is well known that obesity is associated with insulin resistance and an increased risk for type 2 diabetes mellitus. Formerly it was postulated that increased lipolysis and consequently free fatty acid (FFA) production, from with triglycerides overloaded fat

cells, would disrupt glucose homeostasis via Randle's hypothesis. Lipodystrophy, however, also leads to insulin resistance. Recently it has become clear that adipose tissue functions as an endocrine organ and secretes numerous proteins in response to a variety of stimuli. These secreted proteins exert a pleiotropic effect. They include leptin, resistin, adiponectin, acylation-stimulating protein, tumour necrosis factor-alpha and interleukin-6. The stimuli for production and the site and mechanism of action in relation to insulin resistance will be discussed. None of these proteins are, however, without controversy with regard to their mechanism of action. Furthermore, some of these proteins may influence each other via common signalling pathways. A theory is presented to link the interrelationship between these adipocyte secretory products and their effect on insulin resistance (Jazet et.al., 2003).

Non-diabetic obese humans adapt to insulin resistance by increasing beta-cell mass. In contrast, obese humans with type 2 diabetes have an approximately 60% deficit in beta-cell mass. Recent studies in rodents reveal that beta-cell mass is regulated in response to insulin resistance through increased beta-cell supply (islet neogenesis and beta-cell replication) and/or decreased beta-cell loss (beta-cell apoptosis). Prospective studies of islet turnover are not possible in humans. The frequency of beta-cell apoptosis was related to the rate of increase of islet amyloid. These prospective studies suggest that the formation of islet amyloid rather than the islet amyloid per se is related to increased beta-cell apoptosis in a murine model of type 2 diabetes. The current studies also support the concept that replicating beta-cells are more vulnerable to apoptosis, possibly accounting for the failure of beta-cell mass to expand appropriately in response to obesity in type 2 diabetes (Butler et.al., 2003).

Therapies for Diabetes mellitus

Hypoglycemic Drugs

Sulfonylureas

Sulfonylureas work by stimulating insulin release from the beta cells of the pancreas and may slightly improve insulin resistance in peripheral target tissues (muscle, fat). On average, this class reduces glycosylated hemoglobin A_{1c} (HbA_{1c}) levels by 0.8 to 2.0 percent and fasting blood glucose (FBS) concentrations by 60 to 70 mg per dL (3.3 to 3.9 mmol per L), with the greatest reductions observed in patients with the highest FPG concentrations at the initiation of therapy (DeFronzo, 1999). Hypoglycemia is the most worrisome side effect of the sulfonylureas. It is of particular concern with agents that are metabolized to an active metabolite with significant renal excretion. These agents include chlorpropamide (Diabinese) and glyburide, both of which should be avoided in the setting of impaired renal function and used with caution in elderly patients. Glipizide and glimepiride are associated with a lower incidence of hypoglycemia. All sulfonylureas have been associated with weight gain and thus, may not be the optimal first choice for obese patients.

Meglitinides

Repaglinide (Prandin) is a new non-sulfonylurea insulin secretagogue agent, the first available from the meglitinide class. Nateglinide (Starlix), the newest member of the class, closely resembles that of the sulfonylureas. The meglitinides stimulate the release of insulin from the pancreatic beta cells. However, this action is mediated through a different binding site on the "sulfonylurea receptor" of the beta cell, and the drug has somewhat different characteristics when compared with the sulfonylureas. Unlike the

commonly used sulfonylureas, the meglitinides have a very short onset of action and a short half-life. Repaglinide has shown similar effects on HbA_{1c} and FPG levels when compared with glyburide, 0.5 to 2 percent and 65 to 75 mg per dL (3.6 to 4.2 mmol per L), respectively (Luna et.al., 1999). Some potential advantages of this class of agents include a greater decrease in postprandial glucose and a decreased risk of hypoglycemia.

Biguanides

Metformin works by reducing hepatic glucose output and, to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues. Metformin has been shown to reduce HbA_{1c} levels by approximately 1.5 to 2.0 percent and FPG levels by 50 to 70 mg per dL (2.8 to 3.9 mmol per L) (DeFronzo, 1999). Other effects include a reduction in plasma triglyceride levels and low-density lipoprotein (LDL) cholesterol levels.

On the whole, metformin has a favorable side effect profile. Most of the related side effects (including metallic taste, gastrointestinal discomfort and nausea) are transient and commonly reported only during initiation of therapy. Slow-dosage titration is recommended to lessen these effects. Taking the drug with meals may also lessen the severity of the gastrointestinal side effects. Because metformin does not affect insulin secretion, it is not associated with hypoglycemia when used as monotherapy, but can potentiate hypoglycemia when used in combination with a sulfonylurea or insulin. A rare, but more worrisome potential adverse effect is that of lactic acidosis. Metformin is unusual among the oral antidiabetic drugs in that therapy has been associated with a lack of weight gain and even weight loss in some overweight patients (Hermann et.al., 1994). Although these observed effects make it an ideal first-line agent in overweight patients,

results from studies have shown that metformin also improves glycemic control in patients who are not overweight.

Thiazolidinediones

The thiazolidinediones work by enhancing insulin sensitivity in both muscle and adipose tissue and to a lesser extent by inhibiting hepatic glucose production. These agents have a notable effect on improving insulin resistance, particularly when used in combination with other antidiabetic drugs, but have no effect on insulin secretion. Monotherapy with these agents has been associated with a 0.5 to 1.5 percent reduction in HbA_{1c} levels and 25 to 50 mg per dL (1.4 to 2.8 mmol per L) reduction in FPG levels (DeFronzo, 1999). As a class, the thiazolidinediones have also been shown to alter lipid profiles in patients with type 2 diabetes. Results from studies with troglitazone consistently show a decrease in triglyceride levels--in some cases by as much as 33 percent (Saltiel & Olefsky, 1996). The effects on high density lipoprotein (HDL) cholesterol levels have been either favorable or neutral, while some studies report an increase in total and LDL cholesterol levels. Newer data reveal that as monotherapy, rosiglitazone is associated with increases in total, LDL and HDL cholesterol levels and either no change or increases in triglyceride levels. Patients treated with pioglitazone have displayed mean decreases in triglyceride levels, mean increases in HDL cholesterol levels, and no consistent mean changes in LDL and total cholesterol levels. Thiazolidinediones have been shown to interfere with expression and release of mediators of insulin resistance originating in adipose tissue (e.g., increased free fatty acids, decreased adiponectin). Prevention of lipid accumulation in tissues critical to glycaemia such as visceral adipocytes, liver, muscle and beta-cells at the expense of

lipids accumulating at the less harmful subcutaneous site may be central to their net metabolic effect. Moreover, their anti-inflammatory properties also make them interesting in the prevention and treatment of atherosclerosis and possibly other inflammatory conditions (e.g., inflammatory bowel disease) (Stumvoll, 2003).

Alpha-glucosidase inhibitors

Acarbose (Precose) and miglitol (Glycet) are the two agents available in this class. Alpha-glucosidase inhibitors act by inhibiting the enzyme alpha-glucosidase found in the brush border cells that line the small intestine, which cleaves more complex carbohydrates into sugars. Because they inhibit the breakdown and subsequent absorption of carbohydrates (dextrins, maltose, sucrose and starch; no effect on glucose) from the gut following meals, the largest impact of these drugs is on postprandial hyperglycemia. Their effect on FPG levels is modest. They have been associated with a reduction in HbA_{1c} by 0.7 to 1.0 percent and FPG levels by 35 to 40 mg per dL (1.9 to 2.2 mmol per L) (DeFronzo, 1999). Thus, these agents are most useful in patients who have mild FPG elevations or in patients with predominant postprandial hyperglycemia. The most bothersome side effects observed with these agents are gastrointestinal, including abdominal discomfort, bloating, flatulence and diarrhea but are reversible with discontinuation. Therapy with acarbose has been linked to elevations in serum transaminase levels and the use of this agent is contraindicated in patients with liver cirrhosis.

Insulin

Insulin treatment is necessary in case of IDDM to control hyperglycemia and development of the ketoacidosis. Maximum decline occurs in plasma glucose at 30

minute following intravenous insulin administration and at 2-3 hours after subcutaneous insulin administration. Various forms of insulin like rapid acting, intermediate and long acting are commercially available. Insulin administration is also associated with some side effects like hypoglycemic shock, weight gain and an increased risk of atherogenesis (Sinha et al, 1996; USKPD, 1998).

Gene and Islet therapy

Gene and islet transplantation therapy can provide an ideal solution for the treatment of IDDM. Tremendous experimental efforts are in progress to make transplanted islets more viable and functional for the longer period of time. Scientists are trying to make human/non-human engineered insulin producing cells suitable for graft within special immunoisolation barrier membranes. A significant number of animal studies have demonstrated the potential of islet cell transplantation in restoring the normoglycemia in context of immuno-regulation achieved by gene transfer of immuno-regulatory genes to allo- and xenogenic islets ex vivo. Gene and cell therapy is also used to induce tolerance to auto- and allo-antigens and to generate the tolerance state in autoimmune rodent model of type I diabetes. For human diabetics, islet transplantation is still under experimental stage. Successful clinical trails are being conducted with these advance strategies to achieve the final goal i.e. the cure of IDDM. The achievement of gene and cell therapy in type 2 diabetes is less evident. Type 2 diabetes will likely require a better understanding of the processes that determine insulin sensitivity in the periphery (Giannoukakis and Robbins, 2002)

Other Additive Therapies

Exercise

Exercise helps insulin to work better and lower your blood glucose, blood pressure and cholesterol levels. It also strengthens the heart and improves blood circulation and reduces body fat and thus controls body weight.

Vitamin E and α -Lipoic acid

Diabetes produces a state of increased free radical activity. The purported effects of vitamin E on glucose control relate to the vitamin's potent lipophilic antioxidant activity, with possible influences on protein glycation, lipid oxidation, and insulin sensitivity and secretion. Through unknown mechanisms, it may also affect nonoxidative glucose metabolism (Mooradian et.al., 1994) and α -lipoic acid, also known as thioctic acid, a disulfide compound synthesized in the liver is another potent lipophilic antioxidant. It is a cofactor in many multienzyme complexes and may also play a role in glucose oxidation (Konrad et.al., 1999). Experimental in vitro data have shown possible effects in enhancing glucose uptake in muscle and preventing glucose-induced protein modifications.

Certain metals like Chromium, Vanadium, Selenium, Manganese, Zinc and Potassium etc. have also shown the hypoglycemic activities and some of these metals are being used for the treatment of diabetes.

Chromium species

Chromium (Cr³), a trace element in its trivalent form, is required for the maintenance of normal glucose and lipid metabolism. Supplementation with chromium does not appear to reduce glucose levels in euglycemia, but however, have some efficacy

in reducing glucose levels in hyperglycemia (Ryan et.al., 2003). Experimentally, chromium deficiency is associated with impaired glucose tolerance, which can be improved with supplementation (O'Connell, 2001). Most individuals with diabetes, however, are not chromium deficient. In addition to glucose control, the supplement has been studied for its effects on weight control, lipids, and bone density. Its action is linked with glucose tolerance factor (GTF), and has been shown to increase the number of insulin receptors, to enhance receptor binding, and to potentiate insulin action. Some suggest that chromium picolinate is the preferred form because it is utilized more efficiently (Trow et.al., 2000).

Magnesium

Hypomagnesemia is common in patients with diabetes, especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses. Deficiency of magnesium can potentially cause states of insulin resistance (Walti et.al., 2003). Studies have examined magnesium's potential role in the evolution of such complications as neuropathy, retinopathy, thrombosis, and hypertension. Magnesium supplementation in diabetic patients had shown a significant fall in serum cholesterol levels, LDL cholesterol and triglycerides and a rise in HDL cholesterol levels and a reduced insulin-stimulated glucose uptake (Lal et.al., 2003; Djurhuus et.al., 2001). Magnesium is a cofactor in various enzyme pathways involved in glucose oxidation, and it modulates glucose transport across cell membranes. It may increase insulin secretion and/or improve insulin sensitivity and peripheral glucose uptake. It has been shown to have no effect on hepatic glucose output and nonoxidative glucose disposal (Mooradian et.al., 1994). Because it is

an intracellular cation, it is difficult to measure accurately, and total body stores are seldom measured.

Vanadium

Vanadium has been described as either a nonessential nutrient or a nutrient that is required only in minute quantities, as no physiological role of the trace element has yet to be found (Goldwasser et.al., 2000) Human deficiency has not been documented. There are no accurate assays in clinical settings, and there is no recommended daily allowance. Vanadium exists in several valence forms, with vanadyl (+5) sulfate and sodium metavanadate (+4) being the most common supplement forms. Its mechanism of action in glycemic control is thought to be primarily insulin-mimetic with upregulation of insulin receptors. In animal models, it has been shown to facilitate glucose uptake and metabolism and to enhance insulin sensitivity. Clinically, it may enhance glucose oxidation and glycogen synthesis, and it may modulate hepatic glucose output (O'Connell, 2001). An organic vanadyl coordination compound, was reported to concomitantly stimulate insulin secretion *in vitro* from isolated rat pancreatic islets (Conconi et.al., 2003). Vanadium supplementation had also been shown to upregulate GLUT 4 expression in diabetic animals *in vivo* (Mohammad et.al., 2002).

Hypolipidemic drugs

The National Cholesterol Education Program Adult Treatment Panel (ATP) has identified low-density lipoprotein (LDL) cholesterol as the primary target for evaluating and treating dyslipidemia. For many patients with several lipid abnormalities to reach the lipid lowering goals may require the use of combination therapy. Patients with diabetes mellitus are at high risk of cardiovascular diseases. Dyslipidemia is an important risk

factor for cardiovascular complications in diabetes. Increased triglyceride and reduced HDL-cholesterol plasma concentrations are common features of dyslipidemia in type 2 diabetes. The LDL particles are small and dense and have an increased atherogenicity. Abnormalities in lipoprotein composition are observed in diabetes mellitus, especially in type 2. Post-hoc subgroup analyses of studies on the effect of lipid-lowering therapy on cardiovascular events suggest that treatment of dyslipidemia in diabetes may prevent cardiovascular complications. There are increasing indications that dyslipidemia in diabetes mellitus deserves aggressive treatment and that lipid target levels should be very low (Niemeijer-Kanters, 2001). Reducing elevated levels of low-density-lipoprotein cholesterol (LDL-C) significantly reduces the incidence of coronary heart disease (CHD) events and mortality in hypercholesterolemic patients.

Several classes of hypolipidaemic drugs are currently available in the market :

Statins (HMG-CoA reductase inhibitors): Based on their lipid-lowering abilities, safety, and tolerability profiles, Statins or HMG-CoA reductase inhibitors are the first-line pharmacotherapeutic agents for hypercholesterolemia. Widely used to reduce LDL-C and thus blocking regression of atherogenic plaques and reduction of CHD mortality; side effects include myalgia, liver dysfunction. These classes of drugs exert their major effect i.e. reduction of LDL levels through a mevalonic acid like moiety that competitively inhibits HMG-CoA reductase by product inhibition (Alberts et. al., 1980). Statins affect blood cholesterol levels by inhibiting cholesterologenesis in the liver, which results in increased expression of the LDL receptor gene. The greater number of LDL receptors on the surface of hepatocytes results in increased removal of LDL from the blood, thereby lowering LDL-C levels. Statins lower 35% to 45% triglycerides and 20% to 55% LDL-C

and increase 5% to 10% HDL-C levels depending on dose and statin used. Eg. Atorvastatin, Flavostatin and Cerivastatin etc. Hepatotoxicity and myopathy are the commonly found adverse effects of statins.

Bile acid sequestrants (resins) including cholestyramine, colestipol, and colesevelam – low toxicity due to low absorbance, may increase serum triglycerides, not often used. They are highly positively charged and bind negatively charged bile acids. Because of their large size, resins are not absorbed, and the bound bile acids are excreted in stool. Since over 95% of bile acids are normally reabsorbed, interruption of this process depletes the liver's pool of bile acids, and hepatic bile acid synthesis increases. As a result, hepatic cholesterol content declines, stimulating the production of LDL receptors, an effect similar to that of statins.

Nicotinic acid (Niacin) – This is the best agent available for increasing HDL-C (increment of 30% to 40%); it also lowers triglycerides by 35% to 45% (as effectively as fibrates and more potent statins) and reduces LDL-C levels by 20% to 30% (Knopp et al., 1985). In adipose tissue, niacin inhibits the lipolysis of triglycerides by hormone sensitive lipase, which reduces transport of free fatty acids to the liver and decreases hepatic triglyceride synthesis (Grundy et al., 1981). Esterification of fatty acids, and increase apo B degradation (Jin et al., 1999). Two of niacin's side effects, flushing and dyspepsia, limit patient compliance. Hepatotoxicity, produce severe hyperglycemia in diabetes mellitus patients, elevate uric acid levels, atrial tachyarrhythmias, atrial fibrillation, etc. are the other side effects observed. Increased risk of myositis and liver dysfunction when used with HMG-CoA reductase inhibitors.

Fibrates – lower triglycerides, LDL-C and increase HDL-Cholesterol levels; improves glycemic control and often used with HMG-CoA reductase inhibitors. Effects of these compounds on blood lipids are mediated by their interaction with peroxisome proliferator-activators (PPARs) (Kesten et. al., 2000) which regulate gene transcription, fatty acid oxidation, increases LPL synthesis, and reduces expression of apo C-III. An increase in LPL would enhance the clearance triglyceride-rich lipoproteins. A reduction in hepatic production of apo C-III, which serves as an inhibitor of lipolytic processing and receptor-mediated clearance, would enhance the clearance of VLDL. PPAR α also stimulates apo A-I and apo A-II expression (Staels & Auwerx, 1998), which increases HDL levels. Usually well tolerated, gastrointestinal side effects, rash, urticaria, hair loss, myalgia, fatigue, headache, impotence, anaemia, flu like syndrome, renal failure, hepatotoxicity.

MTP inhibitor – MTP (Microsomal Triglyceride transfer Protein) transfer triglycerides and other nonpolar lipids to the apoproteins of nascent lipoproteins as they form in the intestine and liver and is required for the synthesis and secretion of chylomicrons and VLDL. An MTP inhibitor targeted to the liver would decrease VLDL production, thereby decreasing plasma triglyceride levels and ultimately reducing LDL production from VLDL.

Dietary and biliary cholesterol absorption inhibitor – Ezetimibe is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol, resulting in lowered plasma total cholesterol and LDL-C levels (Van, 2000).

ACAT inhibitor – ACAT-1 is expressed in several tissues, including macrophages (Chang et. al., 1993). Avasimibe, an inhibitor of ACAT enzyme, appears to reduce

macrophage and cholesteryl ester contents of lesions in cholesterol-fed rabbits and could affect atherosclerotic lesion development, an effect that could stabilize lesions (Bocan , 2000).

Combination Therapy

Diabetes mellitus is defined as a “metabolic syndrome” and can be precisely said that “type 2 diabetes mellitus represents a syndrome of metabolic and haemodynamic abnormalities as a result of complex pathophysiological interactions between hyperglycemia, β -cell dysfunction, hypertension, insulin resistance, dyslipidaemia, endothelial cell dysfunction and proteinuria which underlie the aetiology and progression of micro- and macro-angiopathy” (Das, 2002). Keeping this in view, combination therapy for diabetes mellitus has become inevitable. In recent years it has been shown that the control of the latter has to be much more strict and that in many cases monotherapy does not achieve the established aims. At the same time, new oral anti-diabetic medicines have recently appeared with different mechanisms of action. The possible combinations of treatment with different oral anti-diabetics, or else with oral anti-diabetics with insulin, are very numerous and have shown their effectiveness in reducing glycemia and the glycosylated haemoglobin. Selection of the type of association will depend on the individual aims of control, on the physiopathological mechanism presumably involved in each case, on the efficacy, cost and secondary effects of each medicine, as well as on the characteristics of each patient. (Menendez, 2002). Several of the available oral agents have been studied in combination and have been shown to further improve glycemic control when compared to monotherapy (Riddle, 2000). Reasonable combinations of agents include a sulfonylurea plus metformin, a sulfonylurea plus an alpha-glucosidase

inhibitor, a sulfonylurea plus a thiazolidinedione, metformin plus repaglinide, biguanide plus alpha-glucosidase inhibitor, and metformin plus a thiazolidinedione.

Combination therapy is a valuable treatment option for patients with persistent lipoprotein disorders and for those requiring more than 1 agent to effectively reach their LDL cholesterol goals. In addition, some physicians may be hesitant to use high doses of a lipid-lowering agent because of the potential for adverse effects. Fibrates are the treatment of choice for reducing triglycerides but, like monotherapy, are not effective for normalizing LDL cholesterol. However, fibrate-statin combinations are widely used to enhance reductions of triglycerides and LDL cholesterol along with increases in HDL cholesterol. Such combination should be avoided in patients predisposed to myopathy because of renal or liver impairment, increased age, debilitated status, surgery, trauma, or heavy exercise. **Ezetimibe** is the first in a new class of lipid-lowering agents known as selective cholesterol absorption inhibitors, which inhibit intestinal absorption of sterols, particularly dietary and biliary cholesterol. An ezetimibe-statin product currently in development may be beneficial for patients who need additional LDL cholesterol lowering despite statin therapy. However, ezetimibe only minimally affects HDL cholesterol and has no significant effect on triglyceride levels; therefore, its use will be limited for patients with multiple lipid abnormalities.

Herbal Therapy

The above class of drugs have their own serious side effects when taken for a long time. Hence interest has generated towards complimentary and alternative therapy. In recent years popularity of complimentary medicine such as dietary measures and traditional herbal therapy, described by ayurvedic and indigenous systems of medicine,

which were commonly used in India (Warner, 1996) has increased. Herbal therapy is one of the complimentary and alternative therapies. Herbal preparations / agents are preferred antidiabetic agents because of their easy availability, more economical and have lesser or no side effects as compared to other drugs. Enormous advances have been made in medical care but more people are still using herbal or alternative remedies. In chronic conditions such as diabetes patients may turn to alternative remedies that have been purported to improve glycaemic control. In a survey, it was seen that Of the diabetic subjects, 78% were taking prescribed medication for their diabetes, 44% were taking over-the-counter supplements and 31% were taking alternative medications. Diabetic subjects spent almost as much money on over-the-counter supplements and alternative medications together as they did on their diabetic medications. (Ryan et.al., 2001). Plant derivatives with purported hypoglycemic properties have been used in folk medicines and traditional healing systems around the world. Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. Among them, metformin was derived from the flowering plant, *Galega officinalis* (Goat's Rue or French Lilac), which was a common traditional remedy for diabetes (Pandey et.al., 1995; Oubre et.al., 1997). Nutritional supplements, botanicals, diet, and lifestyle considerations have application in decreasing insulin requirements and helping to maintain more normal blood glucose levels. In addition, they may prevent the onset of complications of hyperglycemia, including retinopathy, nephropathy, neuropathy and macro- and microangiopathy (Head, 1997).

There are a lot of medicinal plants described for the treatment of diabetes of which only a few are being systematically evaluated. The ethnobotanical information

reports about 800 plants that may possess anti-diabetic potential (Aларcon-Aguilara et.al., 1998). A wide array of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM (Ivorra et.al, 1988; Grover et.al., 2002).

Some of those which have gained credence popularly in the treatment are enumerated below:

Allium species: sativum and cepa – *Allium sativum* (garlic), a member of the lily family, is most commonly used worldwide for flavorful cooking. Much of the clinical literature on garlic has focused on its potential antioxidant activity and microcirculatory effects, e.g., allicin and ajoene for use in hypertension and hyperlipidemia and it is also known for cancer prevention and antibacterial activity (Pareddy and Rosenberg, 1993). Few studies have examined its effects on insulin and glucose handling by allyl propyl disulfide, a volatile oil, and S-allyl-cysteine sulfoxide, a sulfur containing amino acid (Sheela and Augusti, 1992). Although evidence is preliminary, garlic may be useful in diabetes. *Allium cepum* (onion) also contains allyl propyl disulphide and has similar purported hypoglycemic properties. Reported mechanisms of allium species include increased secretion or slowed degradation of insulin, increased glutathione peroxidase activity, and improved liver glycogen storage (Bailey and Day, 1989). Allicin has antibacterial and antioxidant activity. It may possibly increase blood levels of catalase and glutathione peroxidase activity. Ajoene decreases the activity of factors needed for lipid synthesis by reducing the thiol group in coenzyme A and HMG CoA reductase and also by oxidizing NADPH. The only clinical trial available for *Allium cepa* is a small RCT (Randomized Controlled Trial) of allyl propyl disulphide extract capsules from

onion in nondiabetic volunteers ($n = 6$); investigators showed an acute decrease in fasting blood glucose and increase in insulin, supporting an insulin-mediated effect (Augusti and Benaim, 1975).

Aloe vera is the most well-known species of aloe, a desert plant resembling the cactus in the Liliaceae family. It is popularly used to treat burns and promote wound healing. The dried sap of the *Aloe vera* is a traditional remedy for diabetes in the Arabian peninsula (Pandey et.al., 1995), although aloe gel is preferred over the sap as the latter contains the laxative anthraquinone (Yongchaiyudha et.al., 1996). Aloe gel, obtained from the inner portion of the leaves, contains glucomannan, a hydrosoluble fiber which may in part account for its hypoglycemic effects (Shane-McWhorter, 2001). Hypoglycemic effects of Aloe and its bitter principle is mediated through stimulation of synthesis/or release of insulin from the β cells of pancreas (Ajabnoor, 1990). Case reports of five type 2 diabetic individuals reported decreases in fasting blood glucose as well as HbA_{1c} (Ghannam et.al., 1986).

Coccinia indica (ivy gourd) is a creeping plant that grows wildly in many parts of the India subcontinent, and is used to treat "sugar urine" (madhumecha) in Ayurveda, a traditional East Indian healing system. The mechanism of action of *Coccinia indica* is not well understood, but the herb appears to have insulin-mimetic properties (Kamble et.al., 1988; Azad Khan et.al., 1979). The one RCT of this herb ($n = 32$), conducted in India, reported significant changes in glycemic control following 6 weeks' use of powder from locally obtained crushed dried leaves in poorly controlled or otherwise untreated patients with type 2 diabetes (Azad Khan et.al., 1979). Two other open-label prospective trials

offer supporting evidence of a hypoglycemic effect (Kuppurajan et.al., 1986; Kamble et.al., 1998).

Ginkgo Biloba is one of the world's oldest living tree species, dating back more than 200 million years. Extracts from dried leaves of younger trees are used in complementary therapies. Active ingredients include flavonoids (ginkgo-flavone glycosides) and terpenoids, consisting of ginkgolides and bilobalides (Kleijnen and Knipschild, 1992). In diabetes, ginkgo biloba may be of use in ameliorating peripheral circulatory problems, such as intermittent claudication (Pittler and Ernst, 2000). There is also some evidence that it may benefit sexual dysfunction (Cohen and Bartlik, 1998). The flavone glycosides, including quercetin, kaempferol, and isorhamnetin, are thought to have antioxidant activity and inhibit platelet aggregation. The ginkgolides are thought to improve circulation and inhibit the platelet-activating factor. The bilobalides are thought to have neuroprotective properties (Kleijnen and Knipschild, 1992).

Ginseng species – Several different plant species are often referred to as ginseng. These include Chinese or Korean ginseng (*Panax ginseng*), Siberian ginseng (*Eleutherococcus senticosus*), American ginseng (*P. quinquefolius*), and Japanese ginseng (*P. japonicus*). Principal components are believed to be the triterpenoid saponin glycosides (ginsenosides or panaxosides). Hypoglycemic effects have been shown in streptozotocin rat models (Shapiro and Gong, 2002). Ginseng contains a family of steroid-like compounds called ginsenosides. Although there are many subtypes, ginsenosides are tetracyclic triterpenoid saponin glycosides thought to have various hormonal and central nervous system (CNS) effects. Animal research has indicated that

ginseng may lower blood glucose by possibly decreasing the rate of carbohydrate absorption into the portal hepatic circulation (Yuan et.al., 1998) and possibly increasing glucose transport and uptake (Ohnishi et.al., 1996). Another potential mechanism is modulation of insulin secretion (Kimura et.al., 1981). Some ginseng fractions have increased serum insulin levels and glucose-stimulated insulin secretion in mice. Reported mechanisms of action include decreased rate of carbohydrate absorption into the portal hepatic circulation, increased glucose transport and uptake mediated by nitric oxide, increased glycogen storage, and modulation of insulin secretion (Shane-McWhorter, 2001). The available evidence for American ginseng in diabetes suggests a possible hypoglycemic effect. Two longer-term trials administered American ginseng for 8 weeks ($n = 36$ and $n = 24$); both reported decreases in fasting blood glucose and HbA_{1c} (Sotaniemi et.al., 1995; Vuksan et.al., 2001).

Gymnema sylvestre is another commonly used herb in Ayurveda. The plant is a woody climber that grows in tropical forests of central and southern India. According to common folklore, chewing the leaves causes a loss of sweet taste, hence the popular Hindi name of the plant "gurmar," meaning "destroyer of sugar." Early animal studies reported blood glucose-lowering effects in animals with residual pancreatic function, but no effect in total pancreatectomized animals. Studies of an ethanol leaf extract, GS4, in diabetic rat and rabbit models have reported regeneration of islets of Langerhans, decreases in blood glucose, and increases of serum insulin (Shanmugasundaram et.al., 1990). Mechanism of action is unknown; postulated theories include an increase in insulin release through cell permeability, increase in β -cell number, and stimulation of β -cell function (Persaud et.al., 1999). No side effects have been reported secondary to

gymnema use. The hypoglycemic principles like gymnemosides and gymnemic acid were isolated from the saponin fraction of *G. sylvestre* (Murakami et.al., 1996). One study was conducted in type 1 diabetic patients on insulin, 27 took 200-mg gymnema capsules after breakfast and supper and 37 took insulin only for a period of 6–30 months. After 6–8 months, mean HbA_{1c} decreased in the gymnema group from a baseline of 12.8 to 9.5% ($P < 0.001$). After 16–18 months, 22 patients remaining on gymnema had a mean HbA_{1c} of 9% (P values not given). At the end of 26–30 months, six patients remaining on gymnema had a mean HbA_{1c} of 8.2% (P values not given) (Shanmugasundaram et.al, 1990). Another study was conducted in patients with type 2 diabetes on sulfonylureas; 22 took 400 mg/day of gymnema capsules in addition to sulfonylurea treatment, and 25 took a placebo and sulfonylureas for a period of 18–20 months. Mean HbA_{1c} decreased from a baseline of 11.9 to 8.48% ($P < 0.001$). Mean FBG decreased from 174 to 124 mg/dl after 18–20 months ($P < 0.001$). Five patients were able to discontinue sulfonylureas. In this study, lipids also decreased significantly. Patients on placebo had no significant changes in HbA_{1c}, FBG, or lipids (Baskaran et.al., 1990).

Momordica charantia is a vegetable indigenous to tropical areas, including India, Asia, South America, and Africa, also known as balsam pear, karela (karolla), and bitter melon. Active components are thought to be charantin, vicine, and polypeptide-p (an unidentified insulin-like protein similar to bovine insulin). The aqueous extract powder of *M. charantia* was reported to effectively reduce blood glucose in diabetic rats (Virdi et.al., 2003) and appears to have multiple consequences on glucose and lipid metabolism that strongly counteract the untoward effects of a high fat diet (Chen et.al., 2003) and is also reported to show antioxidant activity (Scartezzini and Speroni, 2000). Bitter melon

contains several chemical constituents, including the glycosides mormordin and charantin. Bitter melon also contains the alkaloid mormordicine. Its seeds contain the abortifacients α -mormorcharin and β -mormorcharin, as well as the pyrimidine nucleoside vicine. The specific components thought to contribute to its hypoglycemic activity include charantin, polypeptide P, and vicine. Other theoretical actions include extrapancreatic activity, such as increased tissue glucose uptake, liver/muscle glycogen synthesis, and decreased blood glucose synthesis through depression of the enzymes glucose-6-phosphatase, fructose-1, and 6 bisphosphatase, and enhanced glucose oxidation by enzyme G6PDH pathway. Theoretical mechanisms include increased insulin secretion, tissue glucose uptake, liver muscle glycogen synthesis, glucose oxidation, and decreased hepatic gluconeogenesis. Studies in alloxan-induced diabetic rabbits have suggested hypoglycemic effects (Akhtar et.al., 1981). Two controlled short-term metabolic trials in patients with type 2 diabetes ($n = 18$ and $n = 9$) have reported acute effects on blood glucose with *Momordica charantia* fruit juice, as well as subcutaneous vegetable insulin extract (Baldwa et.al., 1977). An early study of 19 patients (11 with type 1 and 8 with type 2 diabetes) used polypeptide-P zinc chloride, a momordica extract, prepared in the same manner as bovine insulin. This "plant insulin" was injected subcutaneously in five patients with type 1 diabetes and six patients with type 2 diabetes. No details of randomization or blinding were provided. FBG was measured at the time of injection, as well as 4, 6, 8, and 12 h after injection. The control group, consisting of six patients with type 1 and two patients with type 2 diabetes, did not receive any treatment (Khanna et.al., 1981).

Ocimum sanctum (holy basil) is another commonly used herb in Ayurveda (related species include *Ocimum album* and *Ocimum basilicum*). Studies in animal models suggest hypoglycemic effects (Chattopadhyay, 1993) although the mechanism of action remains unknown. Postulated effects include enhanced β -cell function and insulin secretion. Diet containing leaf powder (1%) fed to normal and diabetic rats for 1 month significantly reduced fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglycerides and total lipids (Rai et.al., 1997). The plant has also demonstrated antioxidant (Kelm et.al, 2000) and hypolipidaemic effect (Sarkar et.al., 1994). The one available controlled clinical trial of *Ocimum sanctum* ($n = 40$) showed positive effects on both fasting and postprandial glucose in patients with type 2 diabetes using a local preparation of fresh leaf powder mixed in water for 4 weeks (Agrawal et.al., 1996).

Silbum marianum (milk thistle), a member of the aster family, has been primarily studied for its purported effects on alcoholic and viral hepatitis, rather than for glycemic control. Chemical constituents are found in the fruit, seeds, and leaves. Milk thistle contains silymarin, which is composed of three main constituents: silybin, silychristine, and silidianin. Silybin is thought to have the most potent biological activity (Flora et.al., 1998). However, silymarin is rich in flavonoids, potent antioxidants, and some have postulated a potential benefit for those who have insulin resistance secondary to hepatic damage (Shane-McWhorter, 2001). Mechanisms are based on the herb's antioxidant activity and effects on hepatocyte stabilization with decreased glutathione oxidation, as well as on restoration of normal malondialdehyde concentrations (Velussi et.al., 1997). Milk thistle was evaluated in a 12-month, randomized, open-label trial in 60 type 2 diabetic patients with cirrhosis. All subjects used insulin. One group of 30 received

600 mg/day of silymarin, and the other group of 30 received a placebo for 12 months. Mean FBG declined from 190 mg/dl at baseline to 165 mg/dl at 12 months ($P < 0.01$ vs. baseline). HbA_{1c} declined from 7.9% at baseline to 7.2% at 12 months ($P < 0.01$ vs. baseline). Mean daily insulin requirement decreased significantly from 55 units/day at baseline to 42 units/day at 12 months ($P < 0.01$ vs. baseline). Results were significant in the group of patients on silymarin, but not in the control group (Velussi et.al., 1997).

Many of the plants which are used in day-to-day life also show antidiabetic properties. The commonly used fenugreek or “methi”, turmeric or “haldi”; both of which are an important ingredient of Indian cuisine; emblica or “amla” an important ingredient of major ayurvedic preparations and tonic.

Trigonella foenum graecum Linn. (fenugreek) has been studied, particularly in India, for the treatment of diabetes. An erect 2 to 3 foot tall annual herb with light green leaves and small white flowers and commonly known as fenugreek or “methi” in Hindi. The plant of family Fabaceae, its brownish-yellow seeds has a bitter taste mainly due to furostanol glycosides. Defatted fenugreek seed powder was given to IDDM patients at a dose of 100 grams daily in two divided doses over a 10-day period. The fenugreek-treated group exhibited a 54% decrease in 24-hour urinary excretion of glucose, as well as a reduction in total cholesterol, LDL, VLDL, and triglycerides (Sharma et.al., 1990). Animal studies have also demonstrated the hypoglycemic and hypolipidemic effects of



Elicostemma littorale Blume



Curcuma longa rhizome

Elicostemma littorale



Emblica officinalis fruit



Trigonella foenum-graecum seeds

fenugreek (Khosla et.al., 1995; Petit et.al., 1995). Chemical constituents of the plant include saponins, many of which are glycosides of diosgenin. The seeds also contain the alkaloids trigonelline, gentianine, and carpaine compounds. Other components of the seeds include several C-glycosides. The seeds contain up to 50% mucilaginous fiber. Other seed constituents include 4-hydroxyisoleucine, an amino acid, and fenugreekine. Purported mechanisms include delay of gastric emptying, slowing carbohydrate absorption, and inhibition of glucose transport from the fiber content (Madar, 1984), as well as increased erythrocyte insulin receptors and modulation of peripheral glucose utilization and thus showing pancreatic and extra-pancreatic effects (Raghuram et.al., 1994). Many studies in alloxan-rat models have shown modulated exocrine pancreatic secretion (Sharma, 1986). Various components of the seeds have varying activities. For example, the component called fenugreekine, a steroidal saponin peptide ester, may have hypoglycemic properties. Trigonelline, another component, may exert hypoglycemic effects in healthy patients without diabetes, but other studies have shown that fenugreek has no effect on fasting or postprandial blood glucose levels in nondiabetic subjects (Bordia et.al., 1997). A single trial in type 1 diabetes, have reported improved glycemic control using seed powder incorporated into unleavened bread (Sharma and Raghuram, 1990: Sharma et.al., 1990). Fenugreek seeds has also showed normalization of disrupted free radical metabolism in diabetic animals its supplementation in the diet by decreasing lipid peroxidation and increasing antioxidant status.

Curcuma longa Linn. – Belongs to the family Zingiberaceae and is a perennial herb widely cultivated mainly in India and China. In India, popularly known as “Haldi”. It contains a variety of sesquiterpenes. Assay-guided fractionation of the Ethyl acetate

soluble fraction of the rhizomes of *Curcuma longa* furnished three DPPH free radical scavenging diarylheptanoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin (Song et.al., 2001). Curcumin is the yellow pigment and its derivatives extracted from the rhizome is a potent anti-inflammatory product, has good antioxidant and inhibits lipid peroxidation and maintains the activities of antioxidant enzymes such as – superoxide dismutase, catalase, glutathione peroxidase (Pulla Reddy & Lokesh, 1992) and is also capable of scavenging oxygen free radicals (Pulla Reddy & Lokesh, 1992). Curcumin which showed cytotoxicity to lymphocytes and Dalton's lymphoma cells and experiments indicated that turmeric extract and curcumin reduced the development of animal tumours (Kuttan et.al., 1985). Curcumin also inhibited LPS-induced activation of nuclear factor kappa B and reduced the biological activity of TNF in L929 fibroblast lytic assay (Chan, 1995). The effect of turmeric and its active principle curcumin was found to significantly decrease reduced blood sugar, Hb and glycosylated hemoglobin levels along with oxidative stress encountered by alloxan-induced diabetic rats (Arun & Nalini, 2002) and a distinct tendency to counter change elevated levels of lipid fractions in streptozotocin-induced diabetic rats (Babu & Srinivasan, 1997). Fifty per cent ethanolic extract of *Curcuma longa* (tuber) feeding elevates HDL-cholesterol/total cholesterol ratio and a significant reduction in the ratio of total cholesterol/phospholipids, serum cholesterol and triglycerides (Dixit et.al., 1988). A new, water soluble, 5-kDa peptide--Turmerin--from turmeric (*Curcuma longa*) has been found to be an efficient antioxidant/DNA-protectant/antimutagen. Turmerin forms 0.1% of the dry weight of turmeric and is obtained in a crystalline form. It is a heat stable, noncyclic peptide containing 40 amino acid residues, with a blocked N-terminal and leucine at the C-terminal. Turmerin at 183

vitamin C (Manjunatha et.al., 2001). Evaluation of *Embllica officinalis* (Amla) fresh juice in cholesterol-fed rabbits lowered serum cholesterol, TG, phospholipid and LDL levels by 82%, 66%, 77% and 90%, respectively. Similarly, the tissue lipid levels also showed a significant reduction and aortic plaques were regressed. *E. officinalis* juice treated rabbits excreted more cholesterol and phospholipids, suggesting that the mode of absorption was affected (Mathur et.al., 1996).

***Enicostemma littorale* Blume** – It is a small perennial herb of family Gentianaceae, the whole plant is used for medicinal purposes. It is commonly known as “chota chirayita” in Hindi or “mamejua” in Gujarati. It is very bitter and pungent mainly due to the presence of the alkaloid gentianine and the glycoside swertiamarin (Govindachari, 1966; Natarajan et.al., 1972; Ghosal & Jaiswal, 1980). Secoiridoids and their glucosides like swertiamarin, sweroside, gentiopicroside, erythrocentaurin etc. are widely distributed in gentianaceae (Jensen, 1992). Isoflavone genistein and c-glycoflavones like swertisin, isovitexin, genkwanin, apigenin, swertisin-5-O-glucoside and isoswertisin-5-O-glucoside were also isolated from *Enicostemma hyssopifolium*, a synonym of *E. littorale* (Ghosal and Jaiswal, 1980). Chemical investigation of *E. littorale* by Natarajan and Prasad showed four chloroform soluble alkaloids and one water-soluble alkaloid, two sterols and a volatile oil (Natarajan and Prasad, 1972). Iridoid glucoside, swertiamarin isolated from the same plant showed central nervous system (CNS) depressant and cardiostimulant activity (Bhattacharya et.al., 1976). Also its hypoglycemic activity has been reported by Vyas et. al. (1979) in alloxan induced diabetic rabbits.

Our lab had reported its glucose lowering effect (Vijayvargia et.al, 2000; Maroo et.al., 2003a) and antioxidant effect (Maroo et.al., 2003b) in alloxan-induced diabetic rats. Possible mechanism of glucose lowering effect of aqueous extract of *E. littorale* has also been reported which is associated with potentiation of glucose-induced insulin release through K⁺-ATP channel dependent pathway but did not require Ca(2+) influx (Maroo et.al, 2002). Its effect on increasing insulin sensitivity, normalizing dyslipidemia and providing nephroprotection in streptozotocin-induced (NIDDM) diabetic rats was also reported (Murali et.al., 2002).

Aims and objectives of the present study

Diabetes mellitus is a disease with large number of complications (hyperglycemia, dyslipidemia, oxidative stress) and hence therapy must be designed so as to control these with a combinatorial drug. Combination therapy improves therapeutic effect and has reshaped traditional conception of therapy leading to skillful symptom improvement and enduring function of decreasing blood sugar and thus can enhance therapeutic effect and shorten therapeutic course. It was also discovered that the medicinal properties of many herbs required certain other herbs to be present to act as a catalyst. This in turn, increases patient compliance and efficacy when compared to single therapies for various manifestations. Animal studies (Nishizawa et.al., 1995; Suzuki et.al., 1998) and clinical studies (Angelova, 1984; Mozersky, 1999) are also being carried out with combination of herbs for diabetes mellitus. Oral antidiabetic monotherapies directly address only one defect as their primary mechanism of action, and do not control blood glucose sufficiently well to meet current glycemic targets. An insulin sensitizer and

an insulin secretagogue represent a rational oral antidiabetic combination, as they address the dual endocrine defects of insulin resistance and impaired beta-cell function in type 2 diabetes (Howlett et.al., 2003) along with antioxidative and hypolipidaemic properties. Majority of the herbal products available in the market for the treatment of diabetes are a combination of medicinal plants. The multiple constituent nature of botanical products has made standardization a challenging task. Finally, the existing literature in this area includes a considerable amount of study population heterogeneity. Future research may need to more precisely define targeted diabetic populations with regard to disease classification, severity, optimal adjunctive interventions, and perhaps nutrient deficiencies. It will also be important to further elucidate mechanisms of action so that applicability to type 1 or type 2 diabetes can be clarified. Although biological complementary therapies have been studied in human clinical trials, there are many problems with study design, study endpoints, numbers of patients, and study duration. There is insufficient evidence to recommend generalized use for patients with diabetes.

Studies are going on to evaluate the various herb's efficacy when given in combination. The antihyperglycemic effect of a herbal preparation "antidiabetis" consisting of 10 medicinal plants (Petlevski et.al., 2001), D-400, a herbomineral preparation (Mitra et.al., 1996), Cogent db, a compound herbal drug (Pari and Saravanan, 2002) and "Diabetin", a phytopreparation (Bodnar et.al., 2000) were all reported to significantly reduce blood glucose, glycosylated haemoglobin and increased plasma insulin, total haemoglobin along with antihyperlipidemic and antioxidant effects in alloxan-induced diabetic rats and patients.

As discussed earlier, *Enicostemma littorale*, *Curcuma longa*, *Emblica officinalis* and *Trigonella foenum-graecum* showed antidiabetic properties. The present study was an attempt to further investigate the antidiabetic effects of *Enicostemma littorale* and increase the antidiabetic potency by combining these commonly known antidiabetic herbs (*Curcuma longa*, *Emblica officinalis*, *Trigonella foenum-graecum*) along with *E. littorale* and thus to design a potent antidiabetic therapy combating hyperglycemia, dyslipidemia and oxidative stress simultaneously.

Objectives of the study –

- Clinical evaluation of antidiabetic efficacy of *E. littorale* aqueous extract in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) patients.

- To study the hypoglycemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.

- To study the antioxidant and hypolipidaemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.

- To study the comparative antioxidant and hypolipidaemic effect of aqueous extracts of *E. littorale* and herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in cholesterol fed rats.