Review of Literature
3. REVIEW OF LITERATURE

3.1 Introduction to Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Alberti and Zimmet, 1998). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Pickup and Crook, 1998).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputation, and Charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Glycation of tissue proteins and other macromolecules and excess production of polyol compounds from glucose are among the mechanisms thought to produce tissue damage from chronic hyperglycemia. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral vascular and cerebrovascular disease. Hypertension, abnormalities of lipoprotein metabolism and periodontal disease are often found in people with diabetes. The emotional and social impact of diabetes and the demands of therapy may cause significant psychosocial dysfunction in patients and their families.
The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category (type 1 diabetes), the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category (type 2 diabetes), the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretary response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load.

3.1.1 Epidemiology of Diabetes

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age. These findings indicate that the "diabetes epidemic" will continue even if levels of obesity remain constant.
India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world”. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken (Huizinga and Rothman, 2006). The so called “Asian Indian Phenotype” refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity *i.e.*, higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. This phenotype makes Asian Indians more prone to diabetes and premature coronary artery disease. At least a part of this is due to genetic factors. However, the primary driver of the epidemic of diabetes is the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity as evident from the higher prevalence of diabetes in the urban population. Even though the prevalence of microvascular complications of diabetes like retinopathy and nephropathy are comparatively lower in Indians. The prevalence of premature coronary artery disease is much higher in Indians compared to other ethnic groups. The most disturbing trend is the shift in age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation’s health and economy. Early identification of at-risk individuals using simple screening tools like the Indian Diabetes Risk Score (IDRS) and appropriate lifestyle intervention would
greatly help in preventing or postponing the onset of diabetes and thus reducing the burden on the community and the nation as a whole.

![Bar chart showing estimated number of diabetic subjects in India from 2000 to 2030.](image)

Figure 3.2: Estimated number of diabetic subjects in India. (Huizinga and Rothman, 2006)

3.1.2 Clinical features of diabetes mellitus

**Clinical signs and symptoms**

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, vomiting lethargy much of the time, having sores that are slow to heal, very dry skin and blurred vision. Losing feeling or getting a tingling feeling in the feet. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome.
3.1.3 Etiological classification of diabetes mellitus

A major requirement for the clinical management of diabetes is an appropriate system of classification that provides a framework within which to identify and differentiate its various forms and stages.

3.1.3.1 Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency


This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (Atkinson and Maclaren, 1994). Markers of the immune destruction of the β-cell include islet cell autoantibodies (ICAs), autoantibodies to insulin (IAAs), autoantibodies to glutamic acid decarboxylase (GAD65) and autoantibodies to the tyrosine phosphatases IA-2 and IA-
2β (Baekkeskov et al., 1982; Atkinson et al., 1986; Kaufman et al., 1992; Christie et al., 1992; Schott et al., 1994; Schmidli et al., 1994; Myers et al., 1995; Lan et al., 1996; Lu et al., 1996). One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and B genes, and it is influenced by the DRB genes (Cantor et al., 1995; Huang et al., 1996). These HLA-DR/DQ alleles can be either predisposing or protective.

In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others mainly adults (Zimmet et al., 1994). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years. Many such individuals with this form of type 1 diabetes eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves’ disease, Hashimoto’s thyroiditis, Addison’s disease, vitiligo, and pernicious anemia.

b. **Idiopathic diabetes.**

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type-1 diabetes fall into this category of those who do most are of African or Asian origin. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks
immunological evidence for β-cell autoimmunity and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go (Banerji and Lebovitz, 1989).

3.1.3.2 Type 2 diabetes (ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretary defect with insulin resistance)

This form of diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency (Reaven et al., 1976; Olefsky et al., 1982; DeFronzo et al. 1979; Turner et al., 1979). At least initially and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes and it is likely that the proportion of patients in this category will decrease in the future as identification of specific pathogenic processes and genetic defects permits better differentiation among them and a more definitive subclassification. Although the specific etiologies of this form of diabetes are not known, autoimmune destruction of β-cells does not occur and patients do not have any of the other causes of diabetes.

Most patients with this form of diabetes are obese and obesity itself causes some degree of insulin resistance (Kolterman et al., 1981; Bogardus et al., 1985). Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah et al., 1982). Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection (Butkiewicz et al., 1995; Banerji et al., 1994 Umpierrez et al., 1995). This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes (Harris, 1989; Zimmet, 1992; Fujimoto et al., 1987). Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Fujimoto et al., 1987; Moss et al., 1984; Kuusisto et al., 1994; Andersson and Svaardsudd, 1995; Uusitupaa et al., 1993). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be
expected to result in even higher insulin values had their β-cell function been normal (Polonsky et al., 1996). Thus, insulin secretion is defective in these patients and insufficient to compensate for the insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal (Scarlett et al., 1982; Firth et al., 1986; Simonson et al., 1984; Henry et al., 1986; Wing et al., 1994). The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (Zimmet, 1992; Harris et al., 1995). It occurs more frequently in women with prior gestational diabetes mellitus (GDM) and in individuals with hypertension or dyslipidemia and its frequency varies in different racial/ethnic subgroups (Zimmet, 1992; Fujimoto et al., 1987; Harris et al., 1995). It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes (Newman et al., 1987; Barnett et al. 1981). However, the genetics of this form of diabetes are complex and not clearly defined.

3.1.3.3 Other specific types of diabetes

a. Genetic defects of the β-cell.

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action (Herman et al., 1994; Byrne et al., 1996; Clement et al., 1996). They are inherited in an autosomal dominant pattern. Abnormalities at three genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α (Vaxillaire et al., 1995; Yamagata et al., 1996). A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule (Froguel et al.; 1992, Vionnet et al., 1992). Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which in turn stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the "glucose sensor" for the β-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. A third form is associated
with a mutation in the HNF-4α gene on chromosome 20q (Bell et al., 1991, Yamagata et al., 1996). HNF-4α is a transcription factor involved in the regulation of the expression of HNF-1α. The specific genetic defects in a substantial number of other individuals who have a similar clinical presentation are currently unknown.

Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness (Reardon et al., 1992, Ouwenland et al., 1992, Kadowaki et al., 1994). The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy; encephalopathy, lactic acidosis and stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion (Johns, 1995). Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families and such traits are inherited in an autosomal dominant pattern (Gruppuso et al., 1984, Robbins et al., 1984). The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism (Tager et al., 1979, Haneda et al., 1983, Given et al., 1980).

b. Genetic defects in insulin action.

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes (Kahn et al., 1976, Taylor, 1992). Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries (Ciaraldi et al., 1992; Dunaif et al., 1992). In the past, this syndrome was termed type I insulin resistance (Kahn et al., 1976). Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance (Taylor et al., 1992). The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia. Alterations in the structure and function of the insulin receptor cannot be
demonstrated in patients with insulin-resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the postreceptor signal transduction pathways.

c. Diseases of the exocrine pancreas.

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy and pancreatic carcinoma (Schwartz et al., 1978; Cersosimo et al., 1991; Larsen, et al., 1987). With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion (Phelps et al., 1989; Handwerger et al., 1969). Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications on X-ray (Yajnik. et al., 1992). Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.

d. Endocrinopathies.

Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma) can cause diabetes (Soffer et al., 1961; Jadresic, et al., 1982; Stenstrom et al., 1988; Berelowitz, and Eugene, 1996). This generally occurs in individuals with pre-existing defects in insulin secretion and hyperglycemia typically resolves when the hormone excess is removed. Somatostatinoma and aldosteronoma induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion (Berelowitz and Eugene, 1996; Conn, 1965). Hyperglycemia generally resolves after successful removal of the tumor.

e. Drug or chemical induced diabetes.

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance
(Pandit et al., 1993; O'Byrne and Feely, 1990). In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells (Bouchard et al., 1982; Assan et al., 1995; Gallanosa et al., 1981; Esposti et al., 1996). Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids (Pandit et al., 1993; O'Byrne and Feely, 1990). Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency (Fabris et al., 1992; Shiba et al., 1996).

f. Infections.

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella (Forrestet et al., 1971), although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus and mumps have been implicated in inducing certain cases of the disease (King et al., 1983; Karjalainen et al., 1988; Pak et al., 1988).

g. Uncommon forms of immune-mediated diabetes.

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms (Solimena et al., 1992). Patients usually have high titers of the GAD autoantibodies and approximately one-third will develop diabetes. Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues (Taylor and Lilly, 1992). However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases (Taylor and Lilly, 1992). As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.
h. Other genetic syndromes sometimes associated with diabetes.

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus (Rimoin, 1976). These include the chromosomal abnormalities of Down’s syndrome, Klinefelter’s syndrome, and Turner’s syndrome. Wolfram’s syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β-cells at autopsy (Barrett et al., 1995). Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy and neural deafness.

3.1.3.4 Gestational diabetes mellitus

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy (Metzger, 1991). In the majority of cases of GDM, glucose regulation will return to normal after delivery.

GDM complicates around 4% of all pregnancies, resulting in ~135,000 cases annually (Engelgau et al., 1995). The prevalence may range from 1 to 14% of pregnancies, depending on the population studied (Engelgau et al., 1995). GDM represents nearly 90% of all pregnancies complicated by diabetes (Coustan, 1995). Clinical recognition of GDM is important because therapy, including medical nutrition therapy, insulin when necessary and antepartum fetal surveillance, can reduce the well-described GDM-associated prenatal morbidity and mortality (Langer, 1994). Maternal complications related to GDM also include an increased rate of cesarean delivery and chronic hypertension (Langer, 1994; Magee et al., 1993; Cousins, 1995). Although many patients diagnosed with GDM will not develop diabetes later in life (O’Sullivan, 1964; O’Sullivan et al., 1989; O’Sullivan, 1991; Metzger et al., 1993; Coustan et al., 1993; Kjos et al., 1990). Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester. The criteria for abnormal glucose tolerance in pregnancy was defined as two or more blood glucose values out of four that were greater than or equal to two standard deviations above the mean. These values were set based on the prediction of diabetes developing later in life.
3.1.4 Pathogenesis of Diabetes Mellitus

Understanding the pathogenesis of any disease is of prime importance when considering treatment. Recent breakthroughs in the evaluation and management of diabetes and the availability of new therapeutic regimens make it imperative that the primary care physician be aware of these advances to improve patient care.

3.1.4.1 Pathogenesis of Type 1 diabetes mellitus

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is a chronic autoimmune disease that results from a complex interaction of both genetic and environmental factors (Eisenbarth, 1986). Genetic susceptibility likely is followed by humoral as well as T-cell abnormalities, even though organ dysfunction may be absent or occurring subclinically. It is well known that pancreatic tissue taken from children who have died from diabetic ketoacidosis demonstrates a mononuclear cell infiltration confined to pancreatic islet cells, predominantly CD8+ cells, that are likely involved in beta cell specific destruction, which supports the role of islet cell autoimmunity in type 1 diabetes (Conrad et al., 1994). It also has been shown that a long prodrome is present in exhibit a progressive loss of their ability to secrete insulin in response to physiologic secretagogues. The natural history of this disorder can be subdivided into a series of stages, commencing with genetic susceptibility and ending with disease onset (Fig 3). Several studies have suggested that the use of a combination of immunologic markers to islet antigens (e.g., insulin, GAD and the tyrosine phosphatase IA-2) rather than a single test gives a higher predictive value for type 1 diabetes and provides greater sensitivity without significant loss of specificity (Conrad et al., 1994; Kruglyak and Lander, 1995). Prediction of type 1 diabetes is now sufficiently accurate to allow the design of large clinical trials to study the prevention of the disease process and to provide a better understanding of disease pathogenesis in individuals at risk of developing the disease (Bingley et al., 1994; Pietropaolo et al., 1998).
Review of Literature

(a) Genetic Susceptibility

The application of genome-wide scans has resulted in identification of 218 putative loci, but only linkage to human leukocyte antigen (HLA) loci appears to be unequivocal. Although excitement has been generated by the results of genome-wide scans for type 1 diabetes, careful and rigorous replication in many populations along with association studies is necessary before any attempts are made using either positional cloning or the candidate gene approach to identify potentially elusive sequence variations that could influence genetic susceptibility (Kruglyak and Lander, 1995). Convincing evidence exists that 22 chromosomal regions are associated with and linked to type 1 diabetes. The HLA region on chromosome 6–21 (IDDM1) and the insulin gene region on chromosome 11–15 (ZDDM2) confer susceptibility to type 1 diabetes. The contribution of these 2 loci to familial inheritance is 42% for ZDDM1 and 10% for IDDA42. A rare form of autoimmune diabetes resembling type 1 diabetes occurs in autoimmune polyglandular syndrome type I, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). This syndrome is unique among well-known autoimmune disorders in that it is caused by a single gene pair-homozygosity for defect in the AZRE (autoimmune regulator) gene which is localized on chromosome 21q22.3. This example demonstrates that mutation of a single gene can give rise to an array of autoimmune poly-endocrine disorders, including insulin-requiring diabetes of autoimmune origin. Similarly, autoantibodies

Figure 3.4: Hypothetical stages of autoimmune destruction of pancreatic β cells during the natural history of type 1 diabetes mellitus. (Eisenbarth and Liftery; 1996).
to islet cell antigens such as GAD and islet cell antibodies can occur in patients affected by APECED, as seen in IDDM. The nature of the immune abnormalities in APECED remains elusive.

b. HLA Complex and Susceptibility

Amino acid polymorphism at position 57 on the HLA-DQP chain of the HLA class II molecule could influence interaction among the class II molecule, the peptide antigen, and the T-cell receptor, which in turn could influence the specificity of the immune response to foreign and self-antigens. However, other residues in the DQP chain as well as in the DQa chain also appear to be involved in susceptibility to type 1 diabetes (Pietropaolo and Trucco, 2000). Genetic susceptibility to autoimmune diabetes appears to be strongly conferred by HLA-DQA1*0501-DQB1*0201 and -DQA1*0301-DQB1*0302 haplotypes, both of which are in linkage disequilibrium with the DR3 and DR4 alleles. In many populations the HLA-DQB1*0602 allele is rarely found among patients with IDDM, which suggests that this allele may play a protective role in the disease process. At present, possession of a “protective” DQB1*0602 allele is considered a criterion for excluding first-degree relatives of diabetic patients in clinical trials such as the Diabetes Prevention Trial 1 (DPT-1). This trial has been designed to prevent by effective treatment the progression to type 1 diabetes in individuals considered at high risk of developing the disease.

c. The Insulin Gene (IDDM2) and the Biologic Significance of the Variable-Number-of-Tandem-Repeats (VNTR) Region

The central role of insulin in metabolism and blood glucose homeostasis and its unique distinction as the only known pancreatic beta cell-specific antigen make it a likely candidate for an inherited susceptibility to IDDM. A number of studies have suggested that the insulin gene (INS) VNTR region may play a biologic role in the genetic regulation of insulin expression. The proximity of this polymorphism to the INS transcriptional start site (~400 bas pair upstream) makes this an attractive hypothesis. Nevertheless; the exact function of the INS VNTR region is still a subject of discussion. The insulin promoter and thymic autoantigen expression data are consistent with a proposed general hypothesis that self-tolerance to peripheral proteins develops during thymic selection of T cells (Fig 3); this hypothesis, however, is controversial.
d. Environmental Triggers

Even in genetically predisposed individuals, an environmental agent, such as a virus, may be required for the generation of autoimmunity. For example, the incidence of both type 1 diabetes and multiple sclerosis in a given population changes as these individuals migrate to different regions. Such observations, along with the lower than expected rate of concordance among monozygotic twins, suggest that an environmental factor may play a role in the development of autoimmune responses. In the case of type 1 diabetes, the environmental trigger could be the coxsackievirus (Kruglyak and Lander, 1995). Antigenic similarity between a foreign pathogen (e.g., a virus) and an antigen normally present on or in the target tissue (e.g., an autoantigen) may be involved in triggering the onset of type 1 diabetes. This concept is embodied in the term “molecular mimicry.” The best example of a possible molecular mimicry-induced reaction acting in diabetes etiopathogenesis is that presented by Kaufman and colleagues in which the peptide PEVKEK, an amino acid stretch of the P2-C protein of coxsackievirus B, is shared by the islet antigen GAD65 and may activate T cells to destroy GAD65-expressing cells (Kaufman et al., 1992).

e. Autoimmunity to Islet Antigens

A common characteristic of many autoimmune diseases such as type 1 diabetes is a T-cell and humoral response against multiple target antigens. As autoimmunity in type 1 diabetes progresses from initial activation to a chronic state, there is often an increase in the number of islet autoantigen targeted by T cells and antibodies. This condition is termed “epitope spreading” and involves overexpression of cytokines and other inflammatory mediators. This is a cascading process in that T cells activate additional autoreactive B cells and B cells present additional epitopes from different proteins until there is autoreactivity to numerous autoantigens. In a similar fashion, multiple novel peptides within the same autoantigen can activate T cells. Immunologic diagnosis of type 1 diabetes relies essentially on the detection of autoantibodies to islet antigens in the serum of patients (Bingley et al., 1994). Although their pathogenic significance is still unclear, such antibodies have the great advantage of serving as surrogate markers for specific autoimmune responses and now represent major markers for enrollment in primary intervention trials studying patients with type 1 diabetes.
f. Failure of Immunologic Tolerance to Self-Antigens

Tolerance to self-molecules is established and maintained through complex mechanisms in both the thymus (central tolerance) and peripheral lymphoid organs (peripheral tolerance). One attractive hypothesis is that type 1 diabetes is essentially caused by the failure of negative selection of autoreactive T cells in either the thymus or the periphery or is the result of a breakdown in tolerance to islet cell-specific antigens (Fig 4). Recent evidence suggests that molecules with tissue-restricted expression may also be expressed in the thymus (Pugliese et al., 1997). Genes encoding the IDDM-related islet autoantigens insulin, IA-2, GAD, and the neuroendocrine antigen ICA69 are transcribed in the human thymus throughout fetal life and childhood. There is evidence that variation in the expression of IDDM-related autoantigens in the thymus and peripheral lymphoid organs can affect tolerance to these molecules and in turn influence diabetes risk.

![Diagram showing the development of self-tolerance to peripheral proteins during thymic selection of T cells.](image)

**Figure 3.5:** General hypothesis for the development of self-tolerance to peripheral proteins during thymic selection of T cells (Pietropaolo and Le Roith, 2001).
g. Dysfunction and Loss of Regulatory Cells

Alterations in the number and function of regulatory cells may contribute to the generation of an autoimmune state in type 1 diabetes. Dysfunction or loss of CD1-restricted T cells, T cells with y/S receptors, and CD4+/CD25+ T cells may all theoretically contribute to disease pathogenesis through inefficient suppression of pathogenic autoreactive T cells. For instance, in monozygotic twins who are discordant for diabetes, levels of CD1-restricted T cells appear to be diminished in the affected twin. The antigens that activate regulatory T cells are unknown, and the mechanisms by which these cells exert their effect on immune responses remain unclear.

3.1.4.2 Pathogenesis of Type 2 Diabetes

Although single gene mutations are capable of causing type 2 diabetes by affecting either beta cells or insulin action, the most common form of type 2 diabetes is a heterogeneous disorder caused by a dual defect involving beta cell dysfunction and insulin resistance. The primary defects associated with type 2 diabetes are genetic (with the exact genes yet to be defined) with environmental factors contributing to the disease and increasing the risk of complications. The genetic mutations cause type 2 diabetes in a very small proportion of the population and are commonly a result of autosomal inheritance or inheritance of 2 mutant genes from both parents (Groop and Lehto, 1999). The more commonly seen form of type 2 diabetes is apparently heterogenous and polygenic. Attempts to identify genes that are related to the common form have thus far been unsuccessful. Investigators have used 2 major approaches-candidate genes and positional cloning-to search for these genes. Candidate genes were not found to be the cause of type 2 diabetes. Furthermore, positional cloning has identified some regions of the genome that may be related to the risk of type 2 diabetes, but no strong correlation between specific genes and the disease has yet been convincingly identified (Lindgren and Hirschhom, 2001).
a. Normal Physiology

Normal glucose homeostasis. In the fasting and preprandial states, glucose is utilized primarily by the brain (50%), muscle (25%) and splanchnic organs (25%). Hepatic glucose production accounts for most of this glucose through processes that include glycogenolysis and gluconeogenesis. After a meal, glucose absorption leads to a rise in plasma glucose concentration, which in turn leads to enhanced insulin secretion and suppression of hepatic glucose production, primarily through inhibition of glycogenolysis. The increased levels of insulin in the circulation stimulate glucose uptake Primarily by peripheral tissues such as muscle, as well as by the liver, fat and the gut. Normal fasting plasma glucose levels should be between 60 and 100 mg/dL and 2-hour postprandial levels should be ~140 mg/dL.

b. Insulin secretion

Insulin secretion in response to intravenous glucose shows a 2-phase pattern (Figure 3.6). The first phase, which lasts for ~10 minutes, occurs when stored insulin is rapidly released. The second phase, which lasts as long as the plasma glucose level is elevated, occurs as a result of stored insulin and de novo insulin synthesis. Most of the insulin synthesized in islet cells is stored in vesicles available for rapid release on
stimulation; the major stimulus to the release of this insulin is glucose. Glucose entry into the beta cell generates adenosine triphosphate (ATP), which leads to closure of the potassium-ATP channel, leading in turn to membrane depolarization and the opening of a voltage-dependent calcium channel with the influx of calcium, and resulting finally in insulin secretion. Although glucose is the most powerful stimulus, hormones, free fatty acids (FFAs) and amino acids are also insulin secretagogues and glucose modulates the magnitude of the response of these nonglucose secretagogues. Amino acids may increase intracellular calcium concentration, whereas FFAs mediate insulin secretion via phospholipids accumulation. Other mediators include protein kinase A and protein kinase C (Steiner and James, 1992).

**Mobilization of GLUT4 from intracellular Stores to Cell Surface**

![Diagram showing the mobilization of GLUT4](image)

**Figure 3.7:** Insulin-induced glucose uptake in target tissues (Pietropaolo and Le Roith, 2001).

c. Insulin action.

Insulin exerts its biologic action to regulate blood glucose levels by initially binding to and activating the insulin receptor present on cell surface. Insulin binding to the receptor leads to the generation of multiple cellular signaling pathways, some of which are necessary for increased glucose uptake and metabolism. In the liver, first phase insulin secretion inhibits hepatic glucose production in the early postprandial
phase, primarily by inhibiting glycogenolysis and stimulating glucose uptake and glycogen storage. In muscle and in fat cells, insulin enhances the recruitment of glucose transport proteins (GLUT4) to the cell surface, thereby increasing glucose uptake into the cell in the postprandial state (Shepherd and Kahn, 1999). Glucose is then metabolized in muscle by a specific hexokinase to glucose 6-phosphate, and eventually either glucose utilization occurs by oxidation or glucose is stored as glycogen (Figure 3.8).

d. Factors affecting insulin Secretion and insulin action

Figure 3.8:  Glucotoxicity and lipotoxicity. Both increased circulating glucose and free fatty acids (FFAs) in the circulation negatively affect pancreatic insulin secretion and insulin action, leading to increased hepatic glucose production, reduction in glucose uptake in peripheral tissues, and worsening of diabetes (Pietropaolo and Le Roith, 2001).
e. Glucotoxicity

Hyperglycemia can inhibit insulin gene expression and insulin secretion, particularly by impairing glucose-stimulated insulin secretion (Yki-Jarvinen, 1992). The impairment in glucose transport that is characteristic of the insulin resistance of obesity and type 2 diabetes can worsen as a result of chronic hyperglycemia. Hyperglycemia “down regulates” the glucose transport system by reducing GLUT4 levels (inhibiting synthesis) and by inhibiting the intrinsic activity of GLUT4 proteins. The effects of chronic hyperglycemia can be readily reversed in patients with type 2 diabetes by restoring normal plasma glucose concentrations (Figure 3.8).

f. Lipotoxicity

Chronic effects of elevated FFA levels apparently arise from insulin resistance, which is believed due to enhanced lipolysis in the visceral fat depot. As body weight and fat mass increase, the increasing insulin resistance results in a faster rate of lipolysis. These chronically elevated FFA levels are implicated in both the inhibition of glucose-induced insulin secretion by beta cells and the worsening of insulin resistance at the level of the liver and muscle (Boden, 1997) (Figure 3.8). During the stages preceding type 2 diabetes, beta cell response is also abnormal, even while normoglycemia is maintained. Patients with previous gestational diabetes, polycystic ovary syndrome, obesity, and impaired glucose tolerance (IGT) all exhibit insulin resistance. In these patients normoglycemia is maintained by increased insulin secretion from the pancreas. However, first-phase insulin secretory response is reduced despite the insulin resistance that causes enhanced insulin secretion in response to glucose.

3.1.5 Insulin resistance

Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin. Traditionally insulin resistance in the diabetic is considered to be present when more than 200 units of insulin per day are required to control hyperglycemia and prevent ketoacidosis (Foster, 1983). From physiological view point insulin resistance is considered to be present whenever the therapeutic dose of insulin exceeds the secretion rate of insulin which in normal person is 0.4-0.5 units/kg body
weight per day (Kahn, 1986). For practical purpose most physicians consider patients clinically resistant to insulin when the insulin dose exceeds 2.0 units/kg/day (Koffler et al., 1989). Since insulin travels from the β-cells, through the circulation to the target tissue, events at any one of these loci can influence the ultimate action of the hormone.

3.1.5.1 Causes of Insulin Resistance

Insulin resistance can be due to three general categories of causes (Olefsky & Molina, 1990).

1. Abnormal β-cells secretory product
   A. Abnormal insulin molecule
   B. Incomplete conversion of pro-insulin to insulin

2. Circulating insulin antagonists
   A. Elevated levels of counter regulatory hormones e.g. growth hormone, cortisol, glucagon or catecholamines
   B. Anti-insulin antibodies
   C. Anti-insulin receptor antibodies
   D. Amylin?

3. Target tissue defects
   A. Insulin receptor defects
   B. Post-receptor defects

At the physiological level, obesity, inactivity and aging are common causes of insulin resistance. Although moderate compensatory hyperinsulinemia might be well tolerated in the short term, chronic hyperinsulinemia exacerbates insulin resistance and contributes directly to β-cell failure and diabetes (Pessin and Saltiel, 2000; Shulman, 2000). Importantly, the β-cell failure probably does not arise from overwork but rather from deregulated growth and survival signals that accompany insulin resistant states. The stimulation of glucose metabolism by insulin requires that the hormone must first bind to specific receptors that are present on the cell surface of all insulin target tissues. After insulin has bound to and activated its receptor, "second messengers" are generated and these second messengers initiate a series of events involving a cascade of phosphorylation-dephosphorylation reactions that eventually
result in the stimulation of intracellular glucose metabolism. Initial step in glucose metabolism involves activation of the glucose transport system, leading to influx of glucose into insulin target tissues, primarily muscle. The free glucose, which has entered the cell, subsequently is metabolized by a series of enzymatic steps that are under the control of insulin. Of these, the most important are glucose phosphorylation (catalyzed by hexokinase), glycogen synthase (which controls glycogen synthesis), and phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) (which regulate glycolysis and glucose oxidation, respectively) (Pessin and Saltiel, 2000; Whiterhead et al., 2000).

Post binding defects in insulin action primarily are responsible for the insulin resistance in type 2 diabetes. Diminished insulin binding, when present, occurs in individuals with very mild diabetes and results secondarily from down regulation of the insulin receptor by chronic sustained hyperinsulinemia. In type 2 diabetic patients with overt fasting hyperglycemia, post binding defects are responsible for the insulin resistance. A number of postbinding defects have been documented, including diminished insulin receptor tyrosine kinase activity, insulin signal transduction abnormalities, decreased glucose transport, reduced glucose phosphorylation, and impaired glycogen synthase activity.

The glycolytic/glucose oxidative pathway appears to be largely intact and when defects are observed, they appear to be acquired secondarily to enhanced FFA/lipid oxidation. From the quantitative standpoint, impaired glycogen synthesis represents the major pathway responsible for the insulin resistance in type 2 diabetes and family studies suggests that a defect in the glycolgen synthetic pathway represents the earliest detectable abnormality in type 2 diabetes. Recent studies link the impairment in glycogen synthase activation to a defect in the ability of insulin to phosphorylate IRS-1, causing a reduced association of the p85 subunit of PI 3-kinase with IRS-1 and decreased activation of the enzyme (PI 3-kinase) (DeFronzo, 1997 & Garvey, 1998).

Glucose transport activity in type 2 diabetic patients uniformly has been found to be decreased in adipocytes and muscle. In adipocytes from type 2 diabetic human and rodent models of diabetes, there is a severe reduction in GLUT4 mRNA and protein, and the ability of insulin to elicit a normal translocation response and to activate the GLUT4 transporter after its insertion into the cell membrane is impaired. However,
the physiological significance of the blunted increase in muscle GLUT4 mRNA levels in type 2 diabetic subjects is unclear, since both basal and insulin-stimulated GLUT4 protein levels are normal. Large populations of type 2 diabetics have been screened for mutations in the GLUT4 gene. Such mutations are very uncommon and, when detected, have been of questionable physiologic significance.

3.1.5.2 Treatment of Insulin Resistance

Since insulin resistance is the pathogenic feature of both type I and type II diabetes and an underlying cause of the accompanying cardiovascular risk profile, newer anti-diabetics should be such that they combat insulin resistance. Previously, biguanides were the only class of drugs that could improve the insulin sensitivity. However, recently a newer class, thiazolidinediones also known as glitazones has been shown to enhance the sensitivity of muscle and adipose tissue to the action of insulin (Bailey 2000). They are found to enhance glucose uptake in muscle and adipose tissue, reduce gluconeogenesis and glycogenolysis in the liver. These agents improve sensitivity to insulin by binding to nuclear peroxisome proliferator activated receptors-γ (PPAR γ) which acts in conjunction with retinoid X receptor by de-repression to increase transcription of certain insulin sensitive genes (Spiegelman, 1998). Therapeutic efficacy of PPARgamma agonist has been found to be promising clinically but the first one; troglitazone was withdrawn from the market as result of idiosyncratic hepatotoxicity (Saleh et al., 1999). Although, this has not been observed with rosiglitazone or pioglitazone. Long term benefits versus safety are yet to be observed. Apart from thiazolidinediones, there are several other groups of pharmacological agents projected for therapeutic use in insulin resistance. They can be classified depending on the mechanism of action.

a. Insulin sensitizing agents

Dichloroacetate has been shown to increase oxidative glucose metabolism via stimulation of pyruvate dehydrogenase in the in vitro studies. (Staepoole and Green, 1992). Vanadate has been reported to mimic most of the metabolic effects of insulin. In STZ-treated rats vanadate increased glucose uptake and oxidation in muscle (Shechter, 1990).
b. Inhibitors of fatty acid oxidation

Increased free fatty acid (FFA) levels lead to insulin resistance in skeletal muscle this may be a result of decrease in glucose utilization by inhibiting glucose oxidation via inhibition of pyruvate dehydrogenase and reduction in glucose uptake by inhibiting hexokinase. A decrease in FFA oxidation in patients with NIDDM should therefore have a favorable effect not only on hepatic glucose overproduction, but also on peripheral glucose disposal. Carnitine palmitoyl translocase-I (CPT-I) is responsible for the transport of activated long chain fatty acids across the mitochondrial membrane in hepatocytes. Drugs that inhibit this enzyme could thus prevent the intra mitochondrial oxidation of FFA to acetyl CoA, ketone bodies and nicotinamide adenine dinucleotide (NADH). CPT-I inhibitors include clomoxir, etomoxir and TDGA. TDGA has been reported to decrease glucose levels in IDDM and NIDDM subjects (Hubinger, 1992). However, there have been incidences of cardiac hypertrophy in long term animal toxicology studies with both TDGA and etomoxir (Lee et al., 1982).

c. Beta3 adrenoceptor agonists

β-3 adrenergic receptors are expressed almost exclusively in fat. Treatment with β-3 receptor agonist CL316, 243 results in enhanced sensitivity of both whole body glucose uptake and suppression of hepatic glucose production in insulin resistant obese rodents and humans (Weyer et al., 1998). BRL37344, an active metabolite of BRL35135, is a potent β-3 adrenoceptor agonist. BRL 35135 is reported to increase insulin sensitivity in obese patients with NIDDM (Cawthorn et al., 1992).

d. Inhibitors of gluconeogenesis

Hepatic insulin resistance causes gluconeogenesis and thus the inhibition of enzyme pyruvate carboxylase leading to inhibition of gluconeogenesis could prove beneficial. Phenylalkanoic acid derivatives inhibit gluconeogenesis in hepatocytes at concentrations that do not inhibit cell metabolism (Bressler and Johnson, 1992). Though there has been no human study with these agents, a little success has been achieved in animal models.
e. Inhibitors of lipolysis

Adenosine inhibits lipolysis through its action on adipocytes. This action is probably mediated via the alpha receptor subtype. GR79236 is an analogue of adenosine and specific receptor for alpha receptor subtype. This compound inhibits lipolysis in human abdominal wall adipocytes and decreases plasma non-esterified free fatty acids (Strong et al., 1993). However, this is the only evidence for the association of these changes with significant reduction in plasma glucose levels. However, these agents have a short duration of action and the hypoglycemic response is variable.

f. Aldose reductase inhibitors

The use of aldose reductase inhibitors to enhance insulin sensitivity in diabetes mellitus has been reported. These agents probably act by restoring and preserving intracellular reduced glutathione levels, thereby enhancing formation of insulin receptor mixed disulphide bonds (York, 1988). Tolrestat, Statil, Sorbinil are a few aldose reductase inhibitors.

g. Alpha-glucosidase inhibitors

These agents reduce gastrointestinal breakdown and absorption of carbohydrates. They lower plasma glucose concentration and tend to cause weight loss. Acarbose belongs to this class of drugs and is reported to lower insulin levels and gastrointestinal peptide (GIP) levels (Hoffman and Spengler, 1994). This however would potentiate glucose mediated insulin secretion. In addition, acarbose has other effects on gastrointestinal hormones. Alpha-glucosidase inhibitors might be of value in obese diabetic patients but they can not be useful in normal weight diabetics because of their effects on nutrition.

h. Alpha-2 antagonists and imidazolines

Insulin secretion is normally subjected to tonic suppression via α-2 adrenoceptors the possibility of relieving this suppression with selective α-2 antagonists such as midaglizole and MK-912 has been considered (Kashiwagi et al., 1986). Imidazoline compounds like efaroxan can stimulate insulin secretion independently of an α-2 blockade. There is evidence that this may occur via a closure of K+-ATP channels.
and possibly other K+ channels as well as effects at more distal steps in the control of exocytosis (Hirose et al, 1997). With the newer approaches to the therapeutic benefit in insulin resistance related disorders, a lot remains to be studied and scrutinized. Although it is a long way in the research the day does not seem to be far when suitable pharmacological agents with least untoward effects will be available for the treatment of insulin resistance.
3.2 Diabetes and Obesity

3.2.1 Obesity and insulin Resistance

It is commonly accepted that obesity is a health hazard associated with complications such as non-insulin-dependent diabetes mellitus (Type 2 diabetes), dyslipidemias, hypertension and cardiovascular diseases (Turner, 1992; Barrett-Connor, 1989).

3.2.2 Potential Mechanism of Insulin Resistance in Obesity

Insulin resistance is defined as a diminished response of a target cell or organ to a physiological concentration of insulin. Whereas this definition can be applied to all insulin-responsive tissues (skeletal and cardiac muscle, adipose tissue and liver), the mechanism behind this cellular resistance to insulin action are not necessarily the same. Indeed, whereas the binding and early signaling events involved in insulin action are for the most part identical between all the above tissues, there are notable differences in downstream effectors and intracellular metabolic pathways between muscles, fat and liver cells. Thus, insulin resistance is heterogeneous and can take different forms depending on its site of occurrence.
3.2.3 Obesity-Related insulin resistance in different tissues

Insulin resistance develops in both the liver and peripheral tissues (fat and muscle) in obesity-linked diabetes. However, important tissue-specific differences exist in both its development over time and in its primary causes. Thus, insulin resistance can be detected very early in skeletal muscle and liver, even in non-obese nondiabetic relatives of Type 2 diabetes subjects. In contrast; insulin resistance develops much later in adipose tissue when there is a marked lipid accretion in adipocytes.

Insulin is a critical regulator of virtually all aspects of adipocyte biology and adipocytes are one of the most highly insulin-responsive cell types. Insulin promotes adipocyte triglyceride stores by a number of mechanisms, including fostering the differentiation of preadipocytes to adipocytes and in mature adipocytes, stimulating glucose transport and triglyceride synthesis (lipogenesis), as well as inhibiting lipolysis (Kahn and Flier, 2000). Insulin also increases the uptake of fatty acids derived from circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue. Insulin’s metabolic effects are mediated by a broad array of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as changes in gene expression. Insulin action in adipocytes also involves changes
in gene transcription. The transcription factor ADD-1/SREBP-1c (adipocyte
determination and differentiation factor-1/sterol regulatory element–binding protein-
1c) may play a critical role in the actions of insulin to regulate adipocyte gene
expression (Kim et al., 1998; Shimomura et al., 1999; Foretz et al., 1999), by inducing
genes involved in lipogenesis and repressing those involved in fatty acid oxidation.
The relationship between obesity and insulin resistance is seen across all ethnic
groups and is evident across the full range of body weights. Large epidemiologic
studies reveal that the risk for diabetes, and presumably insulin resistance, raises as
body fat content (measured by body mass index [BMI]) increases from the very lean
to the very obese, implying that the “dose” of body fat has an effect on insulin
sensitivity across a broad range (Colditz et al., 1990). Although this relationship is
seen with measures of adiposity such as BMI, which reflect general adiposity, it is
critical to realize that all sites of adiposity are not equal in this regard. Central (intra-
abdominal) depots of fat are much more strongly linked to insulin resistance, type 2
diabetes and cardiovascular disease than are peripheral (gluteal/subcutaneous) fat
depots (Kissebah and Krakower, 1984). This fact about fat and insulin sensitivity has
not been adequately explained. It is possible that an unknown common factor, either
genetic or environmental, produces both insulin resistance and the central pattern of
regional adiposity and that central obesity does not actually cause insulin resistance.
Alternatively, some biochemical feature of intra-abdominal adipocytes may directly
influence systemic insulin sensitivity. A leading hypothesis in this regard is that intra-
abdominal adipocytes are more lipolytically active, in part due to their complement of
adrenergic receptors. This would increase intraportal FFA levels and flux, which
might inhibit insulin clearance and promote insulin resistance by mechanisms that are
still uncertain (Kahn and Flier, 2000).

Adipocytes express and secrete numerous peptide hormones and cytokines, including
TNF-α, plasminogen-activator inhibitor-1, which helps maintain hemostasis;
angiotensinogen, whose proteolytic product regulates vascular tone and leptin, which
plays a central role in regulating energy balance. Adipose tissue can also produce
active steroid hormones, including estrogen and cortisol (Bujalska et al., 1997;
Deslypere et al., 1985).
3.2.4 Candidate Mediators of Obesity Insulin Resistance in Skeletal Muscle and Fat

Several factors have been postulated to be responsible for the development of peripheral insulin resistance in obesity. There are now several pieces of evidence implicating TNF-α as a candidate mediator of obesity-associated insulin resistance (Hotamisligil and Spiegelman, 1994; Hotamisligil and Spiegelman, 1993). TNF-α is expressed at high levels in the enlarged adipose tissue from virtually all rodent models of genetic obesity as well as in obese humans (Kern et al., 1995; Saghizadeh et al., 1996). The cytokine is also over expressed in muscle cells isolated from Type 2 diabetes subjects (Saghizadeh et al., 1996). Moreover, TNF-α was shown to reduce insulin-stimulated glucose uptake both in vivo and in adipose cells in vitro (Hotamisligil et al., 1994a; Hotamisligil et al., 1994b). Importantly, both experimental neutralization and genetic ablation of TNF-α or TNF-α function were reported to improve insulin sensitivity in various animal models of insulin resistance.

Another molecule that has lately received a great deal of attention is leptin, the protein product of the ob gene. With the exception of the ob/ob mice (which have a point mutation in the leptin gene that encodes a nonfunctional protein), all animal models of obesity and the vast majority of obese humans have increased adipose tissue leptin expression and circulating leptin levels (Girard, 1997; Spiegelman and Flier, 1996). Thus, it has been proposed that hyperleptinemia may contribute to the development of peripheral insulin resistance in obesity (Fig 8). Recent studies have shown that leptin have direct effects on isolated rat adipocytes. Long-term exposure of adipose cells to leptin was shown to impair insulin’s ability to induce glucose transport, glycogen synthase, lipogenesis, antipolysis and protein synthesis (Muller et al., 1997).

Leptin, the product of the ob gene, exerts pleiotropic effects, including profound effects on satiety, energy expenditure, and neuroendocrine function. Severe insulin resistance is a well known feature of deficiency of leptin or its receptor in the ob/ob or db/db mouse strains, and these models were among the first to be investigated for the pathogenesis of insulin resistance in the early 1970s. The result of leptin replacement in ob/ob mice on diabetes and insulin resistance is dramatic. Leptin treatment causes both glucose and insulin levels to fall within hours of administration, before changes...
in either food intake or body weight occur and prolonged leptin has effects on glucose and insulin that exceed those seen in pair-fed ob/ob mice (Halaas et al., 1995). Leptin has a clear insulin-sensitizing effect acutely and also after chronic administration to normal rodents (Halaas et al., 1995; Campfield et al., 1995; Pelleymounter et al., 1995). Some evidence suggests that in tissues including muscle and β cells, leptin promotes lipid oxidation and inhibits lipid synthesis, which would promote insulin sensitivity (Muoio et al., 1997; Shimabukuro et al., 1997). So, one of the future courses for research in the field of insulin resistance might be targeted towards development of leptin sensitizers.

3.2.5 Mechanism of insulin resistance in Liver

Among the factors believed to play a potential role in causing liver insulin resistance, elevated FFA levels have received considerable attention. Intra abdominal obesity is believed to be associated with increased portal FFA flux which may lead to a reduced hepatic insulin extraction, enhanced lipoprotein synthesis and increased gluconeogenesis, all features of insulin resistance (Kissebah and Krakower, 1984; Bjorntorp, 1993; Frayn et al., 1996). The molecular mechanisms behind this FFA-mediated liver insulin resistance are still not fully unraveled. Previous studies reported that elevated ambient FFA levels were associated with impaired insulin cell surface binding to isolated hepatocytes, possibly through an effect of lipid oxidation on the internalization/recycling of the insulin-receptor complex and lower receptor-mediated insulin degradation (Hennes et al., 1990; Sevdberg et al., 1992). No defect in receptor tyrosine kinase (TK) activity was detected in FFA-treated cells. It is unlikely that FFA impairs insulin action in hepatocytes by increasing TNF-α expression like in fat and muscle cell. Indeed, TNF-α induced insulin resistance has been shown to occur in fat and muscle but not in liver (Hotamisligil and Spiegelman, 1993).
3.2.6 Mechanisms of Insulin action in intact Cells

Upon binding to its receptor, insulin induces the autophosphorylation of the β-subunits and the intrinsic activation of the receptor tyrosine kinase activity. The activated receptor increases tyrosine phosphorylation of several docking proteins but it is generally accepted that the acute metabolic actions of insulin on glucose transport are principally mediated by the insulin receptor substrates-1 and -2 (IRS-1, IRS-2) (Quon et al., 1994; Zhou et al., 1997) and also in adipocytes by a recently identified 60 KDa protein named IRS-3 (Lavan et al., 1997).

There is now strong evidence that activation of PI 3-kinase by insulin plays an essential role in mediating the hormone’s metabolic actions. Through, its lipid kinase activity, PI3-kinase phosphorylates the lipid PI on the 3 position of the myoinositol ring, yielding PI-3-phosphate. The enzyme can also use other forms of PI in vivo to yield PI (3, 4) P2 and PI (3, 4, 5) P3. PI 3-kinase is a heterodimer consisting of a regulatory subunit of 85 KDa (p85) that contains two SH2 domains, as well as a catalytic subunit of 100 KDa (p110).

Figure 3.10: Mechanism of insulin action upon binding to insulin receptor (Reaven and Laws, 1999).
Binding of tyrosine phosphorylated IRS proteins to p85 induces a conformation change leading to activation of the PI 10 catalytic activity. It is believed that PI 3-kinase activation by insulin is essential for stimulation of glucose transporter (GLUT 4) translocation and glucose transport. Indeed, several studies have shown that relatively specific inhibitors of PI3-kinase (wortmannin and LY 294002), or overexpression of mutated p85 regulatory subunit lacking the ability to bind and activate the p110catalytic subunit, inhibit insulin-mediated GLUT4 translocation and glucose transport in adipocytes and skeletal muscle cells.

These recent studies suggest that activation of PI 3-kinase activity in the unique intracellular GLUT4-enriched vesicles (or membranes closely associated with these vesicles) is a key event involved in insulin action to stimulate GLUT4 translocation to the cell surface. Importantly, activation of PI 3-kinase is essential not only for the stimulation of glucose transport, but also for other metabolic actions of insulin such as stimulation of amino acid uptake (Tsakiridis et al., 1995) glycogen synthesis (Yamamoto-honda et al., 1995) and antilipolysis (Okada et al., 1994).

One potential target of the PI products that may be involved in glucose transport stimulation by insulin is c-Akt or PKB (Protein Kinase B). c-Akt was identified as a serine/threonine protein kinase that is activated by insulin (Burgering and Coffer, 1995). Importantly, its activation is prevented by prior inhibition of PI 3-kinase with wortmannin (Burgering and Coffer, 1995; Alessi et al., 1996). Both GLUT4 and the ubiquitous GLUT1 glucose transporters are expressed in muscle cells but only GLUT4 is stored in specialized vesicles and translocated by insulin. GULT1 is only present at the plasma membrane in skeletal muscle.

3.2.7 Molecular defects in insulin action in obesity

Inhibition of insulin-receptor signaling molecules

3.2.7.1. Insulin Receptor

Abnormalities in either insulin receptor content or function have been frequently described in obese insulin-resistant subjects. On the other hand a decreased tyrosine kinase (TK) activity of the insulin receptor has been reported in both muscle and fat
cells of obese rodents and Type 2 diabetes individuals (Sinha et al., 1987; Le Marchand et al., 1985). Impaired insulin receptor TK function in muscle and fat cells of insulin-resistant subjects is not related to genetic alternations in the insulin receptor gene. Hence, the altered insulin receptor function is not intrinsic but rather thought to be caused by the presence of other cellular or circulating factors (proteins, metabolites, etc.) in obese insulin-resistant individuals.

In addition to tyrosine phosphorylation, both the insulin receptor and IRS proteins undergo serine phosphorylation, which may attenuate signalling by decreasing insulin stimulated tyrosine phosphorylation and promote interaction with 14-3-3 proteins. These inhibitory phosphorylation provide negative feedback to insulin signalling and serve as a mechanism for cross talk from other pathways that produce insulin resistance. Several kinases have been implicated in this process, including PI-3 kinase, Akt, glycogen synthase kinase (GSK)-3 and mammalian target of rapamycin (mTOR). Recent studies show obesity-induced attenuation of insulin signalling might arise from sequential activation of protein kinase C (PKC) and inhibition of nuclear factor κB (IκB) kinase (Saltiel and Kahn, 2001).

Insulin action is also attenuated by protein tyrosine phosphatases (PTPases) which catalyze the rapid dephosphorylation of the receptor and its substrates. Knockout of PTP 1B leads to increased tyrosine phosphorylation of the insulin receptor and IRS proteins in muscle and improves insulin sensitivity. PTP 1B mice are resistant to diet induced obesity, suggesting the brain as an important site of action. This combination makes PTP 1B as a potential therapeutic target in diabetes and obesity (Saltiel and Kahn, 2001).

3.2.7.2. IRS Proteins and PI 3-Kinase

A potential role for a defective expression or function of IRS proteins and PI 3-kinase in the pathogenesis of insulin resistance has been the subject of intense investigation in recent years. It has been reported that IRS-1 phosphorylation and PI 3-kinase activation by insulin are reduced in adipocytes, skeletal muscles, and liver of animal models of obesity and insulin resistance (Folli et al., 1993; Heydrick et al., 1996). A reduced IRS-1 phosphorylation and associated PI 3-kinase activity upon insulin
stimulation has also been demonstrated in skeletal muscle from Type 2 diabetes subjects (Bjornholm et al., 1997; Goodyear et al., 1995).

3.2.7 3. Glucose Transporters

The potential role of glucose transporters in the insulin-resistant glucose transport of muscle and adipose cells has been reviewed extensively in the past few years (Klip and Marette, 1998; Taskiridis et al., 1995). GLUT4 is by far the predominant transporter protein used by insulin to increase glucose transport in muscle and fat cells. Thus it appears possible that a reduced GLUT4 expression may explain insulin resistant glucose transport that typifies insulin resistant subject. GLUT4 expression is markedly altered in adipose tissue of animals with obesity and type2 diabetes. Several groups also have evaluated the effect of insulin on GLUT4 translocation in skeletal muscle of the obese Zucker rat. Further more it has been suggested that the intrinsic activity of translocated transporters is reduced in muscle of Zucker rat. Thus available data suggest that an impaired GLUT4 translocation is the principal defect responsible for reduced insulin stimulated glucose transport in obese and diabetic animal and humans.

3.2.8 Current therapeutic intervention for obesity related to insulin resistance

3.2.8.1 Sibutramine

It is a sympathomimetic agent and clinically used for treatment of obesity (Danforth, 1999). It inhibits the reuptake of serotonin and nor epinephrine in CNS neurons, resulting in loss of appetite and consequently leading to lower food intake and weight loss. It is having modest long term results (Bray and Greenway, 1999).

3.2.8.2 Orlistat

It is an inhibitor of pancreatic and clinically used for treatment of obesity (Danforth, 1999). It blocks intestinal lipid absorption. Similar to sibutramine it is having modest long term results (Bray and Greenway, 1999).
3.2.8.3 Metformin (Biguanide)

Insulin sensitizer currently used for the treatment of type 2 diabetes mellitus (Moneva, and Dagogo, 2002; Setter, 2003). Increases glucose use mainly by inhibiting of hepatic gluconeogenesis and glycogen breakdown (the exact mechanism of its action remains unknown). There is no consensus on its use in subjects who fulfill criteria for metabolic syndrome-X but do not have diabetes. Facilitates glucose uptake in muscular tissue by increasing the activity of glucose transporters and the synthesis of glycogen. Reduces plasma levels of TGs, LDL-cholesterol, FFAs and increases fibrinolysis.

3.2.8.4 Rosiglitazone (thiazolidinedione)

PPARγ-specific agonist currently used for the treatment of type 2 diabetes mellitus (Camp, 2003). Enhance insulin sensitivity in peripheral muscle and adipose tissue. Lead to modest favorable changes in the lipidemic profile (slight elevation of HDL and lowering of LDL and TGs). There is no consensus on its use in subjects who fulfill criteria for metabolic syndrome-X but do not have diabetes.

3.2.8.5 Pioglitazone (thiazolidinedione)

PPARα and PPARγ agonists. Currently used for the treatment of type 2 diabetes mellitus. There is no consensus on its use in subjects who fulfill criteria for metabolic syndrome-X but do not have diabetes (Camp, 2003). Enhance insulin sensitivity in peripheral muscle and adipose tissue Lead to modest favorable changes in the lipidemic profile (slight elevation of HDL and lowering of LDL and TGs).
3.3 Diabetes and Oxidative stress

Glucose in chronic excess causes toxic effects on structure and function of organs, including the pancreatic islet. Multiple biochemical pathways and mechanisms of action for glucose toxicity have been suggested. These include glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, sorbitol formation, and oxidative phosphorylation. There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might cause beta cell damage. However, all these pathways have in common the formation of reactive oxygen species that, in excess and over time, cause chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis. Diabetes mellitus is a disease characterized by hyperglycemia and is caused by absolute or relative insulin deficiency, sometimes associated with insulin resistance. It has multiple etiologies and segregates into two major forms. Type 1 diabetes is an autoimmune disease in which the patient’s own immune system reacts against islet antigens and destroys the beta cell. Type 2 diabetes is a polygenic syndrome with multiple etiologies rather than a single specific disease. As the hyperglycemia of diabetes becomes chronic, the sugar that normally serves as substrate, fuel, and signal takes on the darker role of toxin. Chronic hyperglycemia is the proximate cause of retinopathy, kidney failure, neuropathies and macrovascular disease in diabetes. The beta cell in type 2 diabetes is also adversely affected by chronic hyperglycemia and in this sense, is also a target for secondary complications. As hyperglycemia worsens, the beta cell steadily undergoes deterioration, secretes less and less insulin, and becomes a participant in a downward spiral of loss of function. This relentless deterioration in cell function caused by constant exposure to supraphysiologic concentrations of glucose is termed glucose toxicity.

3.3.1 Mechanisms of Hyperglycemia-induced Oxidative Stress

In physiologic concentrations, endogenous reactive oxygen species (ROS) help to maintain homeostasis. However, when ROS accumulate in excess for prolonged periods of time, they cause chronic oxidative stress and adverse effects. This is particularly relevant and dangerous for the islet, which is among those tissues that
have the lowest levels of intrinsic antioxidant defenses. Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of vascular, retinal and renal tissues. Considerably less work has been performed using islet tissue. At least six pathways are emphasized in the literature as being major contributors of ROS. Each will be considered briefly.

Figure 3.11: Under physiologic conditions, glucose primarily undergoes glycolysis and oxidative phosphorylation (6). Under pathologic conditions of hyperglycemia, excessive glucose levels can swamp the glycolytic process and inhibit glyceraldehyde catabolism which cause glucose, fructose-1:6-bis-P, and glyceraldehyde-3-P to be shunted to other pathways: (1) enolization and α-ketoaldehyde formation; (2) PKC activation; (3) dicarbonyl formation and glycation; (4) sorbitol metabolism; and (5) hexosamine metabolism (Robertson, 2004).

3.3.1.1 Glyceraldehyde Autoxidation

Glyceraldehyde 3-phosphate is a phosphorylation product formed from glucose during anaerobic glycolysis. The partner product, dihydroxyacetone phosphate, also contributes to intracellular glyceraldehyde concentrations via enzymatic conversion by triose-phosphate isomerase. Thereafter, glyceraldehyde 3-phosphate is oxidized by
glyceraldehyde-phosphate dehydrogenase (GAPDH). Continuance of glycolysis yields pyruvate, which enters the mitochondria where it is oxidized to acetyl-CoA and the processes of the tricarboxylic acid cycle and oxidative phosphorylation begin. One alternative to this classic pathway of glucose metabolism is the less familiar route of α-glyceraldehyde autoxidation (Fig. 10, pathway 1). The potential relevance of this pathway to diabetes mellitus was pointed out by Wolff and Dean (Wolff and Dean, 1987), who emphasized that autoxidation of α-hydroxyaldehydes generates hydrogen peroxide (H$_2$O$_2$) and ketoaldehydes. In the presence of redox active metals, H$_2$O$_2$ can form the highly toxic hydroxyl radical. This pathway, therefore, forms two potentially toxic substances, α-ketoaldehydes, which contribute to glycosylation-related protein chromophore development and the hydroxyl radical, a reactive oxygen species that can cause mutagenic alterations in DNA. Although glyceraldehyde is characteristically thought of as an insulin secretagogue, when present in excess it may also inhibit insulin secretion (Hellman et al., 1974). Long term exposure to high glucose concentrations decreases GAPDH activity in islets (Sakai et al., 2003), which favours excess glyceraldehyde accumulation. Exposure of endothelial cells to 30 mM glucose caused GAPDH inhibition (Du et al., 2003) through the mechanism of ROS-activated poly (ADP-ribosylation) of GAPDH by poly-(ADP-ribose) polymerase. This in turn was associated with intracellular advanced glycation end product (AGE) formation and activation of PKC, the hexosamine pathway, and NF-κB.

3.3.1.2 PKC Activation

Dihydroxyacetone can undergo reduction to glycerol 3-phosphate and acylation and thereby increase de novo synthesis of diacylglycerol, which activates protein kinase C, of which there at least 11 isoforms (Fig. 10, pathway 2). Activation of PKC has many biochemical consequences that relate to microvascular disease in diabetes. PKC activation is associated with increases in TGF-β1, vascular endothelial growth factor, endothelin-1, NAD (P)H oxidase, NF-κB and ROS (Brownlee, 2001; Inoguchi et al., 1992; Ishii et al., 1996).

3.3.1.3 Methylglyoxal, Glycation and Sorbitol pathway

Three reactive intracellular dicarbonyls (glyoxal, methylglyoxal, and 3-deoxyglucosone) form AGEs by reacting with amino groups on intracellular and extra
cellular proteins (Thomalley et al., 1999) (Fig. 10, pathways 3 and 4). AGEs play important roles in the pathogenesis of secondary complications of diabetes, especially with regard to microvascular disease in the retina, nerves, and kidney and likely islets. When GAPDH-mediated catabolism of glyceraldehyde-3-P is impaired, such as in the presence of high glucose concentrations, accumulation of glyceraldehyde-3-P and dihydroxyacetone favors formation of methylglyoxal. Additionally, increased flux along the polyol pathway as a result of hyperglycemia results in aldose reductase-mediated NADPH-dependent reduction of glucose to form sorbitol. Oxidation of sorbitol by NAD$^+$ increases the cytosolic NADH: NAD$^+$ ratio, which tends to inhibit GAPDH activity. This can lead to increased levels of triose phosphates, methylglyoxal, and diacylglycerol. This chain of events is also associated with consumption of NAD$^+$ by activated poly (ADP)ribose polymerase, which can be activated by hyperglycemia via increased production of ROS and DNA strand breaks (Du et al., 2003). Above and beyond the damage that reactive dicarbonyls can cause through enhancement of glycation and the formation of AGEs, the Maillard reaction between carbohydrates and proteins also generates ROS (Wells-Knecht et al., 1995). Thus, hyperglycemia simultaneously enhances both glycative and oxidative stress, which together synergistically contributes to the development of diabetic complications.

3.3.1.4 Hexosamine Pathway

In states of excess intracellular glucose, fructose 6-phosphate via glutamine:fructose-6-phosphate aminotransferase (GFAT) can form glucosamine 6-phosphate and then UDP-N-acetylglucosamine, which supports proteoglycan synthesis and the formation of (O)-linked glycoproteins (Fig. 10, pathway 5). This pathway has been shown to be related to increases in transcription of TGF-β, TGF- β1, and PAI-1 and has been implicated in insulin resistance (Brownlee, 2001; Marshall et al., 1991). Glucosamine infusions in rodents and in humans have been associated with interference with glucose sensing by the beta cell and with insulin sensitivity (Monauni et al., 2000). Adenovirus-mediated over expression of GFAT was reported to impair glucose-stimulated insulin secretion and to reduce expression levels of the insulin, GLUT2, and glucokinase genes (Kaneto et al., 2001). The DNA binding activity of PDX-1, a critical transcription factor for these genes, was also markedly reduced. In these
experiments glucosamine was found to increase hydrogen peroxide levels, and the antioxidant n-acetylcysteine prevented the adverse effects of GFAT over expression.

3.3.1.5 Oxidative Phosphorylation

High glucose concentrations increase the mitochondrial proton gradient as a result of overproduction of electron donors by the tricarboxylic acid cycle, which in turn increase production of mitochondrial superoxide (Du et al., 2000) (Fig. 10, pathway 6). In these experiments, inhibition by Mn-SOD or UCP-1 of hyperglycemia-induced overproduction of mitochondrial superoxide prevented the increases in polyol pathway flux, intracellular AGE formation, PKC activation, and hexosamine pathway activity in endothelial cells. High glucose concentrations were shown to increase mitochondrial superoxide production, proton leak, lower ATP levels, and impaired glucose-induced insulin secretion in islets from wild type but not from UCP-2-knockout animals (Krauss et al., 2003), suggesting that superoxide-mediated activation of UCP-2 could play a role in type 2 diabetes. It has also been reported that a 2 mM glyceraldehyde concentration in 24-h islet incubations increased ROS levels and inhibited insulin secretion, effects that were abrogated by n-acetylcysteine (Takahashi et al., 2004). However, in these studies neither inhibitors of mitochondrial oxidative phosphorylation nor adenovirus overexpression of Mn-SOD prevented the ability of glyceraldehyde to increase islet reactive oxygen species levels. 2 mM glyceraldehyde has been reported to increase intraislet glyceraldehyde concentrations to a level similar to that achieved with 20 mM glucose (Taniguchi et al., 2000). Thus, when the glycolytic pathway is swamped by glucose, it seems likely that both mitochondrial and non-mitochondrial pathways contribute ROS to the glucotoxic process that impairs beta cell function.

3.3.2 Therapeutic targets for oxidative stress in diabetes

Oxidative stress is increased in diabetes mellitus and may play an important role in the pathogenesis of the typical long-term complications of human diabetes, like neuropathy and microangiopathy. Protein glycation and glucose autoxidation can generate free radicals that can catalyze lipid peroxidation. Other potential mechanisms of oxidative stress include the reduction of anti-oxidant defense. There are several members of the antioxidant system. Use of anti-oxidants may improve glucose
metabolism and protein glycation and can prevent the development of diabetic complications.

**Figure 3.12 The antioxidant defense system. (Granot and Kohen, 2004)**

Preventive antioxidants (sequestration of transition metal ions that catalyze free radical reactions)
- Transferrin
- Ceruloplasmin
- Albumin
- Ferritin

Enzymatic anti-oxidants (catalyze the reduction of oxidants)
- Superoxide dismutase
- Glutathione peroxidase
- Catalase
- Paraoxonase
Scavenging antioxidants (electron donors to radicals in which the anti-oxidant is sacrificed)

- Ascorbic acid
- α-Tocopherol
- Thiols (glutathione)
- β-Carotene
- Uric acid
- Flavenoids
- Coenzyme Q (ubiquinone)

Synthetic anti-oxidants

- N-acetylcysteine
- Penicillamine
- Xantine oxidase inhibitors (e.g. allopurinol)
- Probucol
- Deferroxamine
- Butylated hydroxytoluene (a food additive)
3.4 Diabetes and Cardiomyopathy

The existence of a diabetic cardiomyopathy is supported by epidemiological findings showing the association of diabetes with heart failure; clinical studies confirming the association of diabetes with left ventricular dysfunction independent of hypertension, coronary artery disease, other heart disease; experimental evidence of myocardial structural and functional changes. The most important mechanisms of diabetic cardiomyopathy metabolic disturbances (depletion of glucose transporter 4, increased free fatty acids, carnitine deficiency, changes in calcium homeostasis), myocardial fibrosis (association with increases in angiotensin II, IGF-I and inflammatory cytokines), small vessel disease (microangiopathy, impaired coronary flow reserve and endothelial dysfunction), cardiac autonomic neuropathy (denervation and alterations in myocardial catecholamine levels) and insulin resistance (hyperinsulinemia and reduced insulin sensitivity).

The epidemiological link between diabetes mellitus and the development of heart failure, independent of atherosclerotic cardiovascular disease. The increased risk of heart failure persists in the diabetic patients after considering age, blood pressure, weight, cholesterol, as well as history of coronary artery disease (Ho et al., 1993; Kannel et al., 1974). Notably, there is a significant association between diabetes and diastolic dysfunction leading to congestive heart failure in the absence of impaired systolic function (Kitzman et al., 2001; McMurray and Stewart, 2000). More recently, patients with unexplained idiopathic dilated cardiomyopathy were found to be 75% more likely to have diabetes than age matched controls (Bertoni et al., 2003). The association between diabetes and cardiomyopathy was strongest among individuals with microvascular complications of diabetes that parallels the duration and the severity of hyperglycemia.

3.4.1 Cellular Mechanisms of Diabetic Cardiomyopathy

The characteristic metabolic disturbances evident in diabetic states are hyperlipidemia (usually in the form of increased triglycerides and nonesterified fatty acids [NEFAs]) and early hyperinsulinemia followed by pancreatic β-cell failure, leading eventually to hyperglycemia. Type 1 diabetes differs principally from type 2 diabetes in that it is
unaccompanied by a period of hyperinsulinemia and is characterized by early as opposed to late-onset hyperglycemia. Alterations in body mass (obesity) and adipocytokines (leptin, adiponectin) have also been implicated in the cardiovascular pathophysiology observed in diabetes. As such, the effects of increased NEFAs, altered insulin action and hyperglycemia can be considered triggers to the cardiac phenotype in diabetes. An understanding of the cellular effects of these metabolic disturbances on cardiomyocytes should be useful in predicting the structural and functional cardiac consequences. Extensive cellular and molecular studies have identified putative mediators, effectors and targets of these metabolic triggers in the pathogenesis of cardiac dysfunction in diabetes (Figure 3.12). The cellular signaling pathways associated with these metabolic triggers that lead to altered myocardial structure and function.

Figure 3.13: The relationship between diabetic metabolic disturbances (triggers) and the mediators, effectors, and intracellular targets that lead to a diabetic cardiomyopathic phenotype (Poornima et al., 2006).
3.4.2 Increased Nonesterified fatty acids

NEFAs play a critical role in triggering the development of cellular insulin resistance but also have been implicated in the development of myocardial contractile dysfunction. NEFAs play a central role in altering cellular insulin signaling through several mechanisms leading to insulin resistance and compensatory hyperinsulinemia (Shulman, 2000; Birnbaum, 2001; Kim et al., 2001) (Figure 3.13). In turn, hyperinsulinemia is an important trigger to the development of cardiac hypertrophy in diabetic cardiomyopathy. NEFAs induce the activation of atypical protein kinase C (PKC), a serine/threonine kinase that phosphorylates and subsequently activates IκB kinase. IκB kinase phosphorylates serine residues on insulin receptor substrate-1 (IRS-1), inhibiting its ability to bind SH2 domains of the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K), impairing insulin signal transduction (Kim et al., 2001). Although this mechanism is active in skeletal muscle and adipose tissue, it has been less clear whether similar mechanisms are apparent in cardiac muscle. Increases in intracellular NEFAs can also alter insulin signaling without affecting IRS-1/PI3K activation (Figure 3.13). Akt-1 activation is critically dependent on the generation of phosphatidylinositol 3,4,5-triphosphate (PtdIns (3,4,5)P3) to bind the N-terminal pleckstrin domain and activate membrane bound kinases responsible for the phosphorylation of serine and threonine residues on Akt-1 that confer catalytic and regulatory properties (Brazil and Hemmings, 2001; Lawlor and Alessi, 2001; Morisco et al., 2000; Schwartzbauer and Robbins, 2001) NEFAs are natural ligands for the nuclear receptor, peroxisome proliferator-activated receptor (PPAR)α, and can induce the up regulation of the phosphatase and tensin homolog deleted on chromosome (Shulman, 2000) (PTEN) which dephosphorylates PtdIns (3,4,5)P3, preventing the activation of Akt-1 (Schwartzbauer and Robbins, 2001). NEFAs can directly alter myocardial contractility independent of altered insulin action by increasing NEFA flux into the myocardium (Unger and Orci, 2000). Recent evidence (Liu et al., 2001) suggests that increases in fatty acyl coenzyme A (CoA) esters within cardiac myocytes may modulate the contractile state of the myocardium by opening of the KATP channel. Activation of the KATP channel leads to shortening of the action potential and reduces trans sarcolemmal calcium flux and subsequently myocardial contractility. Finally, the increased intracellular accumulation of NEFAs may directly
contribute to cell death under circumstances in which accumulating intracellular NEFAs do not undergo oxidation. The reaction between palmityol-CoA, an intracellular intermediate of NEFAs, and serine leads to the generation of the sphingolipid ceramide, and this reaction may be facilitated by the cytokine, tumor necrosis factor (TNF)-α (Halse et al., 2001). Ceramide can induce cellular apoptosis through the induction of NFκB, caspase 3 activation, and cytochrome c release and can inhibit DNA repair by blocking poly (ADP ribose) polymerase (Zhang et al., 2001). Under these circumstances, increased NEFAs are said to cause lipotoxicity (Unger and Orci, 2000). Although lipotoxicity has been implicated in the reduction in pancreatic β-cell reserves, the relevance of these findings in the myocardium remain controversial. Thus, NEFAs play a central role not only in inducing cellular insulin resistance but also in directly affecting myocardial contractility and, under specific circumstances, in the promotion of cardiomyocyte cell death.

Figure 3.14: Role of NEFA in altered myocardial insulin action (Poornima et al., 2006).

3.4.3 Hyperinsulinemia

Cellular insulin resistance may presage frank diabetes by a decade or more and requires compensatory increases in plasma insulin levels to maintain glucose
homeostasis in the face of impaired cellular insulin action, principally in skeletal muscle and liver (Shulman, 2001). The nature and extent of the cellular insulin resistance may be selective to certain organ systems and may vary in terms of the metabolic, mitogenic, prosurvival, and vascular actions of insulin. Hyperinsulinemia may accentuate cellular insulin action in insulin responsive tissues, such as the myocardium, that do not manifest cellular insulin resistance. In this regard, the mitogenic actions of insulin on myocardium during chronic systemic hyperinsulinemia bear directly the commonly observed finding of cardiac hypertrophy in diabetic cardiomyopathy (Ilercil et al., 2002; Iacobellis et al., 2003; McNulty, 2003). There are at least 3 cellular mechanisms whereby hyperinsulinemia mediates cardiomyocyte hypertrophy (Figure 3.14). Acutely, insulin stimulates growth through the same PI3Kγ/Akt-1 pathway by which it mediates glucose uptake. Akt-1 phosphorylates and inactivates glycogen synthases kinase-3β (GSK-3β), a well-recognized inhibitor of nuclear transcription governing the hypertrophic process via the nuclear factor in activated lymphocytes (NFAT-3) (O’Neill and Abel, 2005; Morisco et al., 2005). In addition, Akt-1 activates the mammalian target of rapamycin (mTOR) that activates the p70 ribosomal subunit S6kinase-1, leading to increased protein synthesis (Khamzina et al., 2005; Manning, 2004; Shah et al., 2004; Tremblay and Marette, 2001). However, these mitogenic actions mediated through the insulin receptor may be mitigated when insulin signaling through the PI3Kα/Akt-1 pathway is impaired during chronic hyperinsulinemia. However, chronic hyperinsulinemia may augment myocardial Akt-1 activation indirectly through increased sympathetic nervous system activation (Morisco et al., 2005; Kern et al., 2005; Grassi, 2004; Yosefy et al., 2004). Recent evidence suggests that chronic Akt-1 activation in cardiac myocytes is mediated through β2-adrenergic receptors via protein kinase A and Ca\(^{2+}\)-calmodulin dependent kinase (CaMK), (Morisco et al., 2005) and these mechanisms may predominate when insulin signaling is attenuated through the PI3Kα pathway. In addition, there are other insulin-mediated, but Akt-1–independent, pathways that may be operative, most notably the extracellular signal-regulated kinase (ERK)/mitogen-activated protein (MAP) kinase pathways (Wang et al., 2004; Naito et al., 2003). Significant cellular evidence exists for an insulin-induced activation of the p38 MAP kinase pathway, (Wang et al., 2004) as well as prenylation of both Rho and Ras in the setting of hyperinsulinemia, leading to myocyte hypertrophy and expansion of the extra cellular matrix (Figure 3.15). These redundant pathways provide a strong
mechanistic basis for the development of cardiac hypertrophy associated with chronic hyperinsulinemia as an early accompaniment of type 2 diabetes, even though the glucoregulatory effects of insulin are attenuated.

3.4.4 Hyperglycemia

The mechanism whereby hyperglycemia mediates tissue injury through the generation of reactive oxygen species has been elucidated largely through the work of the Brownlee and colleagues. (Du et al., 2003; Nishikawa et al., 2000; Nishikawa et al., 2000) Hyperglycemia leads to increased glucose oxidation and mitochondrial generation of superoxide (Nishikawa et al., 2000; Cai and Kang, 2001; Farhangkhoee et al., 2003). In turn, excess superoxide leads to DNA damage and activation of poly (ADP ribose) polymerase (PARP) as a reparative enzyme. (Du et al., 2003) However, PARP also mediates the ribosylation and inhibition of glyceraldehyde phosphate dehydrogenase (GAPDH), diverting glucose from its glycolytic pathway and into alternative biochemical pathways that are considered the mediators of hyperglycemia induced cellular injury. These include increases in advanced glycation end products (AGEs), increased hexosamine and polyol flux, and activation of classical isoforms of protein kinase C. Taken together, these data provide mechanistic evidence linking hyperglycemia to altered expression and function of both the ryanodine receptor (RyR) and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2) that may contribute to decreased systolic and diastolic function. In addition, hyperglycemia contributes to altered cardiac structure through posttranslational modification of the extra cellular matrix.
Figure 3.15: Alternative pathways whereby compensatory hyperinsulinemia contributes to myocyte hypertrophy through the sympathetic nervous system activation and MAP kinase/ERK pathways at a time when insulin receptor mediated Akt-1 activation (Poornima et al., 2006).

3.4.5 Stages of diabetic cardiomyopathy

Diabetic cardiomyopathy appears to consist of two major components, the first being a short-term, physiological adaptation to metabolic alterations, whereas the second represents degenerative changes for which the myocardium has only limited capacity for repair. Thus, therapies during the early stages of diabetes can potentially delay or impede the progression of more permanent sequelae. However, it should be noted that many factors such as treatments, metabolic characteristics, lipid profile, and other individual differences may affect the process of development of diabetic cardiomyopathy, and not all diabetic patients are affected by the same factors or to the same degree, which may result in marked variability in the clinical manifestations of the diabetic cardiomyopathy.
3.4.5.1 Early stage

Diabetic cardiomyopathy is initiated by hyperglycemia at an early stage and characterized by metabolic disturbances such as depletion of GLUT4, increased FFAs, carnitine deficiency, calcium homeostasis changes, and insulin resistance. This stage of diabetic cardiomyopathy has insignificant changes in myocardial structure (such as normal LV dimensions, wall thickness and mass) or only substructural changes in myocytes. Cardiac dysfunction usually can only be detected by sensitive methods such as strain, strain rate, and myocardial tissue velocity. Endothelial dysfunction occurs at an early stage.

3.4.5.2 Middle stage

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

3.4.5.3 Late stage

The further changes in metabolism and development of myocardial fibrosis result in myocardial microvascular changes. This stage of diabetic cardiomyopathy is characterized by both myocardial microvascular structural and functional changes probably accompanying recurrent microvascular spasm. Changes in cardiac structure and function are obvious. Diabetic cardiomyopathy at this stage is frequently associated with hypertension and early development of ischemic heart disease in diabetes.
3.4.6 Therapeutic Implications of Diabetic Cardiomyopathy

The mechanisms of metabolic disturbances, myocardial fibrosis, microvascular disease, CAN, and insulin resistance in diabetic cardiomyopathy imply that various treatments might be effective for preventing or delaying the development of diabetic cardiomyopathy and its complications. These include improving diabetic control; use of calcium blockers, angiotensin-converting enzyme (ACE) inhibitors, or related drugs; exercise training; lipid-lowering therapy; antioxidant, and insulin-sensitizing drugs. Hyperglycemia increases levels of FFA, oxidative stress, and growth factors and causes abnormalities in substrate supply and utilization, calcium homeostasis, and lipid metabolism, so diabetic control might be expected to be the most basic and important strategy for preventing development of diabetic cardiomyopathy. Unfortunately, there are scant data to support this expectation. This may be in part due to the differing pathophysiolgies of type I and type II diabetes. (Hansen et al. 2002) showed that type I diabetic patients have impaired myocardial function and perfusion in the basal state, which can be improved by replacement of C-peptide. In eight type I diabetic patients, tissue velocity and perfusion were reduced compared with control subjects, and administration of C-peptide led to improvements in both function and perfusion. The role of poor diabetic control (associated with lower IGF-I levels) in diabetic cardiomyopathy is supported by experimental work showing that exogenous IGF-I treatment can restore the diabetes-induced decline in SERCA and may ameliorate contractile disturbances in cardiomyocytes from diabetic animals (Hansen et al., 2002).

Intracellular retention of calcium in diabetes is associated with depletion of high-energy phosphate stores and a derangement of ultrastructure and cardiac dysfunction. Calcium channel blockers are capable of reversing the intracellular calcium defects and preventing diabetes-induced myocardial changes. Verapamil has been shown to significantly improve the depressed rate of contraction and rate of relaxation, lower peak LV systolic pressure, and elevate LV diastolic pressure (Afzal et al., 1988), as well as to improve the altered myofibrillar ATPase activity, myosin ATPase, myosin isoenzyme distribution, and sarcoplasmic reticular Ca$^{2+}$-pump activities in streptozocin-induced diabetic rats (Afzal et al., 1989). Diltiazem has been shown to suppress interstitial fibrotic changes in type II diabetic mice (Shimada, 1993) and
and these may be lowered by exercise training, resulting in improved myocardial sarcoplasmic reticulum function and vascular function. In addition, exercise training improves cardiac output (DeBlieux et al., 1993) and reverses the changes in contractile properties of the heart in streptozotocin-diabetic rats (De Angelis et al., 200). Improvements in cardiac function are also mediated by decreasing the severity of the diabetic state (Paulson et al., 1992). Improving vascular endothelial dysfunction by exercise training may also play an important role. However, whereas low-intensity exercise training seems to improve cardiovascular function, some types of endurance training may further decrease the reduced myocardial Ca$^{2+}$-activated ATPase and β-adrenergic receptor number in diabetes (Belcastro et al., 1985).

Sympathetic cardiac hyperinnervation can occur concurrently with denervation in diabetic neuropathy and could potentially cause arrhythmia and sudden death. Direct evaluation of myocardial sympathetic innervation has shown the correlation of autonomic dysfunction with diabetic control. The long-term poor glycemic control is associated with the progression of LV adrenergic denervation (Ziegler et al., 1998). However, even in the early stage of diabetes, cardiac sympathetic denervation is only partially reversed with improved metabolic control (Schnell et al., 1997; Burger et al., 1999).

The role of oxidative stress in the development of CAN suggests that antioxidants may have beneficial effects on the cardiac autonomic nervous system through a decline in oxidative stress. The chronic vitamin E administration improves the ratio of cardiac sympathetic to parasympathetic tone in patients with type II diabetes, which might be mediated by a decline in oxidative stress (Manzella et al., 2001). This is supported by antioxidant studies in diabetic animals using vitamin E (Ustinova et al., 2000) and acetyl-L-carnitine treatment (Lo et al., 2000) as well as in diabetic patients using lipoic acid (Androne et al., 2000; Ziegler et al., 1997; Ziegler and Gries, 1997; Haak et al., 1999). In addition, ACE inhibitors such as quinapril have also been shown to significantly improve CAN in diabetic patients (Kontopoulos et al., 1997). Similarly, aldose reductase inhibitors have demonstrated clinical improvement not only in CAN but also in cardiac performance. The effect on cardiovascular performance of sorbinil was studied in patients with diabetic autonomic neuropathy who were free of atherosclerotic coronary artery disease. After 1 yr of treatment,
significant improvement was demonstrated in both the resting cardiac output and the maximal cardiac output, suggesting that the use of an aldose reductase inhibitor may be useful in treating suboptimal cardiovascular performance in patients with diabetic CAN (Roy et al., 1990). Furthermore, decreased MIBG uptake and increased norepinephrine content in diabetic myocardium were completely prevented by insulin therapy started immediately after streptozotocin injection and partially, but significantly, by aldose reductase inhibitor administered immediately after streptozotocin injection. Heterogeneous MIBG distribution also disappeared with the aldose reductase inhibitor therapy. In contrast, diabetic rats treated with insulin or aldose reductase inhibitor therapy that was started 4 wk after streptozotocin injection showed no improvement in MIBG uptake, suggesting the importance of early intervention (Kurata et al., 1997).

Insulin resistance, hyperinsulinemia, atherogenic dyslipidemia, hypertension, abdominal obesity, and impaired hemostasis are risk factors for cardiovascular disease. Insulin resistance results from a combination of genetic and environmental factors and contributes to type II diabetes mellitus. Obesity is associated with insulin resistance, and weight loss has been shown to correct insulin resistance in diabetes (Williams et al., 2003; Dixon et al., 2003), implying that weight reduction through lifestyle modifications (low-calorie diet and exercise) and antiobesity drugs (orlistat, sibutramine, etc.) is the first step of treatment of insulin resistance. Metformin and the thiazolidinediones are used to treat insulin resistance and work through different mechanisms. Metformin reduces FFA efflux from fat cells, thereby suppressing hepatic glucose production, and indirectly improving peripheral insulin sensitivity and endothelial function. In contrast, thiazolidinediones improve peripheral insulin sensitivity by reducing circulating FFAs but also by increasing production of adiponectin, which improves insulin sensitivity. Thiazolidinediones also improve endothelial function and may prevent or delay the onset of diabetes (Stolar, 2002). Combination oral hypoglycemic therapy may be ideal for maintaining adequate glycemic control and improving insulin resistance in patients with type II diabetes. In obese type II diabetic patients inadequately controlled on metformin alone, addition of the insulin-sensitizing agent rosiglitazone improves glycemic control, insulin sensitivity, and β-cell function to a clinically important extent (Jones et al., 2003). Combination of pioglitazone and metformin has also shown to significantly improve
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insulin sensitivity as compared with metformin monotherapy in patients recently diagnosed with type II diabetes (Pavo et al., 2003). Troglitazone has been shown to reduce plasma insulin levels and restore coronary circulation by improving insulin resistance in type II diabetic patients (Sekiya et al., 2001). In addition, weekly intravenous IGF-I bolus therapy has been demonstrated to be effective in inducing sustained insulin sensitivity in a patient with type I diabetes mellitus and massive insulin resistance (Usala et al., 1994). Finally, both ACE inhibitors and exercise may be beneficial for improving insulin resistance. In skeletal muscle, exercise has been demonstrated to recruit a separate pool of GLUT4 to that activated by insulin (Ploug et al., 1998). This leads to an additive effect of insulin and exercise on glucose uptake. Furthermore, exercise increases skeletal muscle GLUT4 gene and protein expression (Goodyear and Kahn, 1998). Should similar changes occur in cardiac muscle, it is conceivable that exercise may have a direct beneficial effect on myocardial function also. The improvement of insulin resistance may result in improvement of cardiac function. Glipizide has been shown to reduce the degree of insulin resistance in the myocardium and improves cardiac function in diabetes. In a neonatal streptozotocin-induced rat model of type II diabetes, animals treated with glipizide for 1 yr exhibited improved myocardial contractile function relative to the vehicle-fed or ad libitum-fed diabetic animals. Heart rate was significantly elevated, and there was a tendency for both the rate of relaxation and contractility to be elevated in the glipizide-treated group (Schaffer et al., 1993). Finally, improvement in insulin sensitivity of cardiac muscle may have benefits other than improved energy utilization. Insulin and IGF-I share multiple intracellular signaling pathways, and both receptors mediate antiapoptotic effects. Improvements in the signaling of these molecules may have an effect to preserve cardiomyocyte number.
3.5 Recent advances in therapy of diabetes mellitus

As we have discussed diabetes is characterized by high concentrations of glucose in the blood, which is caused by decreased secretion of insulin from the pancreas and decreased insulin action. This condition is prevalent worldwide and is associated with morbidity and mortality secondary to complications such as myocardial infarction, stroke and end-stage renal disease. The importance of tight control of blood glucose in either preventing or delaying the progression of complications is recognized. Currently, there are many therapeutic options to treat hyperglycemia in diabetes. However, tight control is difficult to achieve and is often associated with side-effects. Furthermore, no pill or injection to date addresses the problem of dying pancreatic β-cell, a fundamental dysfunction seen in diabetes. Recent advances in understanding insulin secretion, action and signaling have led to the development of new pharmacological agents. Following discussion will provide review of new molecules that are promising candidates for the future management of diabetes, focusing on their mechanism of action, efficacy, safety profile and potential benefits compared with pharmacological agents that are available currently.

3.5.1 Current anti-diabetic drugs: (Goyal et al., 2005)

1. Insulin Preparation
2. Oral Hypoglycemic Agents
   (A) Sulfonylureas e.g. Tolbutamide, Chlorpropamide, Glibenclamide, Glipizide
   (B) Biguanide e.g. Phenformin, Metformin
   (C) Thiazolidinediones e.g. Troglitazone, Ciglitazone, Pioglitazone, Euglitazone
   (D) α – glucosidase inhibitors e.g. Acarbose, Miglitol

3.5.2 Novel drugs at an advanced level of development (Nourparvar et al., 2004)

- Insulin sensitizers: PPARγ agonists
- Hormones: Glucagon Like Peptide-1: -Analogs of GLP-1
  - Inhibitors of metabolism
- Insulin secretagogues: Novel sulfonylureas
  - Meglitinide analogs
3.5.3 Are currently available drugs from modern medicine adequate?

With the discovery of insulin by Banting and Best in 1921, it was hoped that diabetes mellitus would become an easily treatable disease (Rosenfeld, 2002). However, the early expectations that Insulin would "cure" diabetes-mellitus proved disappointing because the prolongation of survival of diabetic persons was accompanied by the development of degenerative complications including retinopathy, renal failure, neuropathy and arterial and cardiac disease. Although insulin appears to be prime factor responsible for diabetes, 90% of diabetics seem to suffer from non-insulin dependent diabetes mellitus (NIDDM) (Kannel, 1985). Patients with NIDDM may have few or none of the classic symptoms of diabetes when first diagnosed. They are not dependent on exogenous insulin for survival and are not prone to the development of ketoacidosis. In general clinical practice, patients with NIDDM are treated with two group of drugs discovered before 1950 i.e. sulfonylureas (glibenclamide, glipizide, glyclazide etc.) and bigunides (phenformin, metformin). However, an anti-diabetic agent that could maintain normoglycemia for longer duration (3-5 years) in diabetic patients remained a challenge (The diabetic controlled and complications trial research group, 1995). On long-term therapy with sulfonylurea, a NIDDM patient may require injection of insulin for adequate control of glucose level. Further, in spite of anti-diabetic therapy patients may still suffer from dyslipidaemia with increase in circulating triglycerides, very low-density lipoprotein (VLDL) and hence an increased morbidity and mortality due to diabetes induced cardiovascular complications (Winocour et al., 1989). The prevalence of hypercholesterolemia (≥ 6.5 mmmol/lit) is considerably higher in both IDDM (27%) and in NIDDM (50%). Levels of HDL-cholesterol tend to be lower in diabetics that matched non-diabetic subjects (Durrington, 1993). This led to the emergence of the concept of Insulin Resistance.
Metformin became the popular drug useful as insulin sensitizer. Diabetes is now defined as the 'Metabolic Dyslipidemic Cardiovascular Syndrome'. In 1996, in search for the drugs useful in insulin resistance especially associated with dyslipidemia, thiazolidinediones like troglitazone, pioglitazone, rosiglitazone were introduced and some of them are still being used. The need for the development of different strategies in the investigation of newer anti-diabetic drugs still remains. The efficacy of currently used antidiabetic drugs is compromised in several ways. Individual oral agents act only on a part of the pathogenic process of diabetes mellitus and hence they may not produce any cure and may not prevent all the complications of diabetes mellitus (Bailey and Turner, 1996; Lebovitz, 1999). They do not reinstate normal insulin sensitivity or normal β-cell function. In addition, these agents do not prevent gradual β-cell loss and hence their usefulness depends upon a critical mass of functional β-cells remaining. Thus, although existing classes of anti-diabetic agents offer a variety of actions that can be combined in a complementary and additive manner, few patients maintain the recommended targets for the good glycemic control, and a normal physiological pattern of glucose homeostasis is rarely reinstated. This emphasizes the urgent need for newer and better therapeutic approaches. Synthetic hypoglycemic agents can also produce serious side effects including hematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy (Larnner, 1985). Therefore the search for more effective and safer antihyperglycemic agents has become an area of current research. Compared to synthetic drugs, herbal preparations are frequently considered to be less toxic with fewer side effects (Momin, 1987). The search for improved anti-diabetic drug therapies must take account of the multiplicity of endocrine and metabolic disturbances and attendant risks and complications of diabetic state. Under these circumstances herbal drugs are now looked upon as an alternate therapy for the treatment of diabetes mellitus. In many countries it is traditional to use herbal medicine to control diabetes.
3.6 Drug discovery from natural products

3.6.1 The Legacy of the Past

For thousands of years, the products of nature, solely, supplied the medicines for human ills. In earlier times, all drugs and medicinal agents were derived from natural substances, and most of these remedies were obtained from higher plants. Today, many new chemotherapeutic agents synthetically derived, based on “rational” drug design.

The research for these therapeutic agents began in the 1780s with the work of Scheele on the organic acids of plants. Bioactive principles were earnestly sought after in the very early part of the nineteenth century, when, in a period of fifteen or so years, the investigation of several removed medicinal plants led to the discovery of a number of the most significant biologically active alkaloids. Some of these alkaloids, morphine, atropine, papaverine and codeine, subsequently became the cornerstones of many aspects of drug discovery today (Sneader, 1985; Foye et al., 1995). Structural modifications of natural products, with a view to enhancing activity or selectivity and reducing side effects or toxicity, developed as organic chemistry grew in the late 19th century (Sneader, 1885). Aspirin was one of the earliest of these chemically modified natural products and recently celebrated 100 years as a commercial entity (Reisch, 1997).

Crude extracts of plants or marine organisms are subjected to screening through bioassays based on specific therapeutic targets. The extracts showing activity are then fractionated by bioactivity-directed fractionation. This is the process whereby the compounds responsible for a given activity in a natural product extract are isolated and characterized. As the compound proceeds through the various stages of the development process, it will be modified through semi-synthesis with a view to enhancing potency, reducing toxicity or modifying solubility. If the biologically active domain within the molecule, i.e. the pharmacophore, can be identified, synthetic process around the pharmacophore may be initiated by the medicinal chemists in order to achieve the same purposes. Only in rare instances does the isolated natural product itself serve as a “magic bullet”.

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3.6.2 The Reality of the present

Farnsworth and colleagues indicated (Farnsworth et al., 1985) that globally there are over 120 compounds from over 90 plants which are used as entity medicinal agents. Significantly, 77% of these were obtained as a result of examining the plants based on their ethnomedical uses and are employed in a manner that approximates those uses. In fact, half of the best-selling pharmaceuticals in 1991 were based on a natural product precursor or pharmacophore (O’Neill and Lewis, 1993).

Despite that record of productivity, natural products drug discovery was deemphasized in many big pharmaceutical companies in recent years. The trend began in the early 1990s, and the reasons were primarily practical. When automation, robotics and personal computers came into the drug discovery scene around the mid-1980s, chemistry became the rate-limiting step in drug discovery programs. The situation worsened in the early 1990s, with the advent of high-throughput screening, fast personal computers, and the breakneck pace at which molecular biology was identifying biological targets, Chemists couldn’t supply the huge numbers of compounds required by screens that by this time were taking months instead of years. At the same time, natural products drug discovery was still being done the traditional way; the process was slow, inefficient and labour intensive and it did not guarantee that a lead from screening would be chemically workable or even patentable (Maureen, 2003). While pure-compound natural product libraries seem to represent the only practical alternative, they are considered uneconomical, mainly because of high production costs. Moreover, the development of optimization technologies for refining such compounds identified in the screens, into successful drugs is still in its infancy (Brohm et al., 2002).

In case, if the active principal can be isolated from the hit extract, and interest continues in the compound, then a recollection of the plant may be needed. However, in 40% of the cases the originally observed biological activity is not reproducible on recollection (Cordell, 2000). Resupply of source material is another problem for both technical and political reasons. Either the raw material is not constantly available in sufficient amounts or political objections claim that biological resources have to be protected (Muller-Kuhrt, 2003). On the other hand, even of the active compound can be obtained from the large scale recollection of a plant or a marine organism, the yield
may be adequate to supply material for chemical modification in order to investigate
the structure activity relationship, enhance potency, etc., prior to a decision with
respect to development. In some instances, the active compound can be synthesized
by the chemist. However, most of the biologically active natural products from plant;
fungal and marine sources are often very complex in structure and possess many
chiral centers. Hence such compounds are often refractory to synthesis, or at least
difficult to produce in a timely manner and in adequate quantities for additional
studies (Cordell, 2000).

Another impediment for natural products in drug discovery is the rigid selection of the
substances based on the ‘Lipinski’s Rule of Five’ or related catalogs of parameters
defining how a promising synthetic molecule should look, though Lipinski himself
stated that his ‘Rule of Five’ is not applicable to natural products (Lipinski
et al., 1997). Additionally, natural products can get companies entangled in sticky
intellectual property issues, Negotiating agreement that are fair to all concerned
parties to develop natural products collected in foreign countries has become
extremely difficult (Maureen Rouhi, 2003). For these reasons, pharmaceutical
companies became reluctant to use natural products as an essential component of their
drug discovery programs. Meanwhile, introduction of combinational chemistry in the
early 1990s was a severe setback to natural products drug discovery. The former
wasn’t only faster and cheaper, it also had the great advantage of clarity with respect
to intellectual property.

As per the current paradigm of drug discovery, it is estimated (Kuhlmann, 1997) that
it requires the evaluation of 50,000-100,000 compounds in order to obtain a single
marketable drug. Not all ‘leads’ will yield a drug. Nearly all (49 of 50) of the
compounds which show promise at an early stage in the development process will fail
when into more advanced animal models. Some compounds will produce unwanted
side effects in humans, or simply may not work, or may not offer improvements over
existing products. Hence there is the need to evaluate large numbers of samples
against each screen. Pharmaceutical companies are now typically working large
number of screening at least a million samples per year using high-throughput
screening (HTS) (Kuhlmann, 1997). On the other hand, it is estimated that a chemist
can synthesize 200-300 compounds per year, or possibly isolate and characterize 100-
150 natural products per year. Thus the conventional synthesis and natural products collectively cannot fulfill the requirement of extensive compound libraries needed for HTS. The belief that combinatorial chemistry can supply wide variety of synthetic chemicals for a given screen in the initial stage of the discovery process inspired the drug companies to eliminate or scale down the efforts involving natural products in drug discovery program (Coredell, 2000).

Recently, an analysis of compounds from combinatorial libraries, natural and drugs in the market was conducted to evaluate how they occupy a statistically defined chemical space (Feher and Schmidt, 2003). It is suggested that combinatorial libraries that mimic the distribution properties of natural products might be more biologically relevant (Maureen Rouhi, 2003).

Till 2002, not a single de novo synthetic molecule coming from combinatorial chemistry has been approved as a drug. However, compounds that have been optimized by combinatorial chemistry are in all phases of drug development. Thus, combinatorial chemistry may not yield much as a discovery tool, but it is excellent for further development of lead molecules. This argument is supported by the alarming decline in the number of new chemical entities in the past decade, from an average of 30 or so to as few as 17, which correlates well with the decreased interest in natural products drug discovery and increased interest in combinational chemistry. The trend is not supporting in as much as natural products have been proven sources of drugs.

Imagine if we eliminated natural products from drug discovery in the past, we would not have the top selling drug class today, the statins, the whole field of angiotensin antagonists and angiotensin converting enzyme inhibitors; the whole area of immunosuppressives; nor most of the anticancer and antibacterial drugs. Imagine all of those drugs not being available to physicians or patients today.

For decades, natural products have been a wellspring of drugs and drug leads. According to a recent survey, 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981-2002 can be traced to or were inspired by natural products. These include natural (6%), natural product derivatives (27%), synthetic compounds with natural product-derived pharmacophores (5%), and
synthetic compounds designed on the basis on knowledge gained from a natural product (Newmann et al., 2003).

In certain therapeutic areas, the productivity is higher. 78% of antibacterials and 74% of anticancer compounds are natural products, have been derived from or inspired by a natural product. These numbers are not surprising if it is assumed that natural products evolved for self-defense, but the influence of natural products is significant even in therapeutic areas for which they might not seem relevant, such as cholesterol management, diabetes, arthritis and depression (Maureen, 2003).

Many researchers are of the opinion that natural products are not relevant to many human diseases. According to this view, natural products are logical starting points in the search for drugs against infections diseases and cancer, since they evolved for self-defense. But why should they be relevant to say schizophrenia? Of course, it is an undeniable fact that natural products have not evolved to interact with human proteins specially. But the point is natural products have evolved to interact with some proteins and those proteins may not be so different from human proteins. By looking for natural and building libraries around them, there are more chances of finding compounds that show activity versus compounds that are just out there but have not evolved in relation to proteins.

Moreover, when one has no idea where to begin in a drug discovery program, nature is a good starting point. It would be unlikely that nature had not yet seen a structure similar to what we need, even if the compound isn’t curing schizophrenia in a plant or a fungus.

Another important advantage of natural products is that they have ‘a biological history.’ Biosynthesis of natural products involves repeated interaction with modulating enzymes, and the actual biological function of many natural products comprises binding to other proteins. Thus, the ability of natural products to interact with other molecules, an indispensable prerequisite to making an unsurprising, but often overlooked, fact that many natural products exhibit advanced binding characteristics compound with synthetics. Most probably, the serially more complex structure of natural contributes to this (Muller-Kuhrt, 2003).
Moreover, natural products offer a diversity of structures which simply cannot be matched through even the most active imaginations of the synthetic organic chemists. It makes a lot of sense to be guided by natural products, as well as nonnatural compounds with validated biological relevance. One may find something sooner or later, but the approach towards drug discovery is intellectually more satisfying than the random screening of compound libraries against a variety of targets.

3.6.3 The Hopes of the Future

With the advent of combinatorial synthesis, some companies considered that this technique would answer the question of how to biologically evaluate a large number of compounds with the minimum of effort. However, the euphoria has subsided. It is now widely recognized that combinatorial chemistry probably does not go well with the primary screening process, but has, however, a very significant role to play when the lead has been identified and potency, bioavailability or some other attribute requires optimization (Ecker and Crooke, 1995).

The title is turning as the limitations of combinational synthesis and rational drug design have sparked a renewed interest in natural products for drug discovery. This interest is stimulated by new technologies for extracting and identifying compounds from complex mixtures and by the results of research in academic institutions and specialist biotechnology companies.

Currently, it is a frustrating and challenging time in the pharmaceutical industry. It is important for them to revisit their decisions in light of new information. As they decide how to allocate limited resources, decision-makers have to view natural products in a new light. They can not waste a lot of resources making a lot of molecules that don’t work. It is important to be aware that natural products drug discovery isn’t like what it used to be. Today, high-quality, pure and resuppliable natural products are becoming available. Thus, problems related to extract screening such as redundancy of compounds can be eliminated. Furthermore, certain suppliers are now able to deliver fully structure-elucidated, high-purity natural product libraries at reasonable prices (Bindseil et al., 2001). Combinational libraries based on natural products have also become available (Tan et al., 1998), enabling promising natural scaffolds to be either produced entirely synthetically by combinatorial chemistry
(Brohm et al., 2002; Nicolaou et al., 1997) or derivatized with synthetic side chains to increase the ‘leadability’ of a molecule. As each interesting natural product can be used as origin for a new library, it is likely that several collections of high-potential test molecules will soon be available. Natural products should no longer be viewed as unapproachable, old fashioned substances rather they should be recognized as accessible, highly variable test molecules.

3.6.4 Traditional systems of medicines using herbal drugs

WHO has approved the use of traditional medicines as a part of the health program for the treatment of diabetes mellitus. To pursue research in these systems of medicine, several USA agencies and institutions such as FDA and National Institute of Health have setup separate wings. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for the primary health care needs. In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine. The potential of plant as a source for new drugs is yet to be explored systematically. Among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically (Verpoorte et al., 1998). A popular herbal shop in East Los Angeles, herbs of Mexico, which serves a large Mexican American population, markets a capsule that contains 18 plant products used in Mexico as hypoglycemic. Practitioners of traditional Chinese medicine, believing that individual herbs work in concern with others herbs for a given benefit, prepare remedies using mixtures of various plant products (Bensky and Gamble, 1986). In India, herbal agents in current use for diabetes were indicated for this purpose in Ayurvedic medicinal texts within some 2,500 years ago (Ajgaonkar, 1979). India has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. In India, a sound knowledge of use of plants for medicinal purpose has come from Unani, Ayurveda and Siddha Systems of Medicine and these systems are still being practiced in all parts of the country. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different
healthcare systems, the evaluation of the rich heritage of the traditional medicine is essential.

**Unani**

Unani medicine owes its origin to Greece. In this system, diseases are considered as a natural process and its symptoms are the reaction of the body to the diseases. Unani system is based on humoral theory. There are several humors in the body like Dam (Blood), Bhalgham (Phlegm), Safra (Yellow bile), and Souda (Black bile). Unani system believes that every person has a unique humoral constitution, which represents his healthy state. Any change in his state affects his health. There is a power of self-preservation or adjustment called 'medicatrix naturae' or defense mechanism (Borins, 1987), which strive to restore disturbances. If this power weakens, imbalance in humoral composition occurs and causes diseases. Diabetes may also be considered as disturbances in humoral system resulting from or leading to disturbances in the post receptor mechanisms of insulin resistance. The Unani medicines help the body to regain this power to an optimum level and thereby restore humoral balance and thus retaining health. Various types of treatments are prescribed in Unani system of medicine. There are: (a) Regimental therapy: includes Diaphoresis, Diuresis, Turkish bath, massage, emesis, purging etc. (b) Diet therapy: aims at treating certain ailments by administration of specific diets or by regulating the quantity and quality of food. (c) Pharmacotherapy: deals with the use of naturally occurring drugs mostly herbal.

**Ayurveda**

Ayurveda is one of the major traditional medicinal systems from India. The word “Ayurveda” means “science of life”. The basic concept of diagnosis and drug development in Ayurveda is based on Tridosha (three major components of disorders) theory, which includes Vayu, Pitta and Kapha. Vayu (Vata) explains the entire biological phenomenon being controlled by the functions of central and autonomous nervous system. Pitta is the manifestation of energy (Tejas) in the living organisms that helps digestion, assimilation, tissue building, heat production, blood pigmentation, activities of endocrine glands and so on. Kapha implies the function of thermotaxis or heat regulation and also the formation of various preservative fluids e.g. mucus. There are three types of diabetes (Madhumeh is the word used for
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diabetes in Ayurveda): Kapha Prameh, Pitta prameh and Vata prameh. Vata prameh correlates well with type I or long term complications of diabetes. Pitta prameh is more or less identical to type 2 diabetes and Kapha prameh is like beginning with obesity as the common denominator. Many Ayurvedic drugs and other Indian plants with curative properties soon came under some sort of scrutiny. Such investigations have continued to the present day and are being reviewed continuously. The term Siddha comes from ‘Siddhi’ means attainment of perfection. This system is almost akin to Ayurveda. This system describes 96 principal constituents of human beings, which include physical, physiological, moral and intellectual components of individuals. When there is any imbalance or slight deviation with these 96 units, diseases occurs. The Siddha medicine consists of psychosomatic system where attention is given to minerals and metals rather than plant constituents. The use of metals and minerals form an integral part of Siddha system of therapy to cure diseases.

3.6.5 Linking of various systems of medicine

The above mentioned traditional systems of medicine have their uniqueness no doubt but there has to be a common thread running through these systems in their fundamental principles and practices. Further, we also believe that there has to be a common link with the principles of modern medicine with these systems of the medicine. Use of the herbs is the common thread for various traditional systems. In many countries it is traditional to use medicinal plants, either a single herb or a polyherbal formulation, to control diabetes. The antihyperglycaemic effect of several plants extracts or herbal formulations that are used as a antidiabetic remedies has been confirmed (Sharma et al., 1992). A database of natural hypoglycemics collected by researchers in Mexico lists almost 800 plants (Lozoya 1994). Researchers in India have documented the use of 150 plants in families with reported hypoglycemic activity (Handa and Chawla, 1989). A recent cross-cultural compendium cites 1,200 medicinal plants used for diabetes (Marles and Farnsworth 1995). Hundreds of products are marketed in India as “natural” agents for lowering blood sugar and decreasing long term complications. These include antibetic, Alphabetic, Diabets, DB-7, Diabetica, Diabetiks, Dia-Comp, DiaVite, GlucoCare, Glucotize, GlycoNase, SugarMax, and Sugar Loss. These formulations are typically the combination
products containing the individual components presented here along with others. However, in order to link them with the principles of modern medicine we must understand the molecular, cellular and molecular mechanism involved in the anti-diabetic activity of these drugs. Under these circumstances, one must consider that herbal drugs may not contain directly the insulin and even if there is protein substance, it can not be absorbed in total from gastrointestinal tract. Herbal drugs have to be considered as the sensitizers of insulin or possessing insulin like substances. We should not forget that the most important drugs biguanides like phenformin, metformin were discovered from a plant Galega officinalis or goat’s rue.

An attempt has been to first classify the herbal anti-diabetics based on the molecular mechanism investigated by various researchers. Table 3.1 gives the classification of some herbal anti-diabetics based on the mechanisms of action. We also classified various herbal drugs based on the chemical nature and their known anti-diabetic related properties (Table 3.1 1). Finally, the attempt has been to suggest various methods for the study of cellular and molecular mechanisms of action as related to anti-diabetic activity.
3.7 Classification of herbal anti-diabetics as per the mechanism of action (Goyal et al., 2007).

3.7.1. Drugs acting like Insulin
Momordica charantia.

3.7.2. Drugs that increase insulin secretion from beta-cells
Panax ginseng (release of insulin from cells) Pterocarpus marsupium, Gymnema sylvestre, Morus bombycis (regeneration of beta-cells) Lythrum salicaria, Trigonella foenum-graecum, Cassia tamala & Swertia chirayata (increase circulating insulin levels).

3.7.3. Drugs inhibiting glucagon secretion
Ke-Tang-Ling.

3.7.4. Drugs that reduce absorption of glucose from gastrointestinal tract
Cyamopsis tetragonolobus, Cuminum nigrum, Andrographis paniculata, Pterocarpus marsupium, Ocimum sanctum, Saccharum officinarum.

3.7.5. Drugs that increase uptake of glucose by muscle
Swertia chirayata.

3.7.6. Drugs inhibiting aldose-reductase activity
Paeonia latiflora, Glycyrrhiza glabra, Aralia elata, Atractyloides lanata, Phellodendron amurense, Acer ginnula, Cinnamomum cortex, Illicium religiosum, Comus macrophylla.

3.7.7. Drugs that increase glucose utilization/ glycogen formation
Gymnema sylvestris, Nelumbo nucifera, Bryonia alba, Lythrum salicaria, Aralia elata, Allium sativum, Panax ginseng, Hygrophila longifolia, Coccinia indica, Swertia chirayata.

3.7.8. Drugs that increase glucose uptake by lipocytes
Ocimum sanctum, Swertia japonica.

3.7.9. Drugs that increase uptake of GLUT-4 in skeletal muscles
Sangbackpitang, a preparation containing Morus bombycis, Panax ginseng, Liriope muscari Pueraria thunbergiana, Poria cocos, Dioscorea batas, Cinnamomum casssia Glycyrrhiza uralensis.

3.7.10. Drugs that inhibit gluconeogenesis
Bauhinia megalandra, Trigonella foenumgraecum, Syzygium cumini.
Table 3.1 Chemical constituents and its mechanism of action

<table>
<thead>
<tr>
<th>Class</th>
<th>Constituents</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole</td>
<td>Indole-3-acetic acid, Indole-3-propionic acid</td>
<td>Inhibit insulinase</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Catharanthine, leurosine, Lochnerine, Vindoline, Tetrahydroalstonine, Vindolinine</td>
<td>Increase ATP content, Increase in lactate/pyruvlate ratio</td>
</tr>
<tr>
<td>Steroid glycosides</td>
<td>β-sitosterol-D-glucoside, 5-25 stigmastadien-3-β-ol-D-glucoside</td>
<td>Inhibition of glucose uptake</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Quinolate, 3-mercaptoepicolinate</td>
<td>Hepatic gluconeogenesis from lactate or alanine</td>
</tr>
<tr>
<td>Sulfur containing compound</td>
<td>Allylpropyl disulphide, Diallyl disulfate oxide</td>
<td></td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>Chromium, Vanadium</td>
<td>Insulin sensitizers, Insulin like activity</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Hypoglycin A, Hypoglycin B</td>
<td>Inhibit β-oxidase enzymes, Blocks oxidation of long chain fatty acid, Increase utilization of glucose</td>
</tr>
<tr>
<td>Guanidine</td>
<td>Galegine</td>
<td>Succinic dehydrogenase and cytochrome oxidase inhibitors</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Nicotinic acid</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Trigonella, Scopoletin</td>
<td>Hepatotoxicity</td>
</tr>
<tr>
<td>Complex carbohydrate</td>
<td>Galactomannans</td>
<td>Inhibition of intestinal glucose absorption</td>
</tr>
<tr>
<td>Indolizidine alkaloid</td>
<td>Castanospermine, Moranoline</td>
<td>Inhibition of α-glucosidase</td>
</tr>
<tr>
<td>Glycans</td>
<td>Aconitan A, Ganoderma B, Panaxans A, E</td>
<td>Potentiation hepatic phosphofructokinase, Glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>Forskolin</td>
<td>Increase intracellular cAMP</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Epicatechin, Quercetin, Myricetin</td>
<td>Inhibit tyrosine kinase</td>
</tr>
<tr>
<td>Peptides</td>
<td></td>
<td>Insulinomimetic</td>
</tr>
<tr>
<td>Phorbol esters</td>
<td></td>
<td>ATP dependent enhancement of glucose stimulated insulin secretion</td>
</tr>
</tbody>
</table>
3.8 Introduction to gallotanin

3.8.1 Vegetable tannin

Plants accumulate a wide variety of secondary compounds, including alkaloids, terpenes and phenols. Although these compounds apparently do not function in primary metabolism such as biosynthesis, biodegradation and other energy conversions of intermediary metabolism, they do have diverse biological activities ranging from toxicity to hormonal mimicry and may play role in protecting plant from herbivory and disease.

Phenolic metabolism in plants is complex and yields a wide array of compounds ranging from the familiar flower pigments (anthocyanidins) to the complex phenolics of the plant cell wall (lignin). However, the group of phenolic compounds known as tannins is clearly distinguished from other plant secondary phenolics in their chemical reactivities and biological activities.

Traditional use of tannins as agents for converting animal hides to leather ("tanning") is one manifestation of the most obvious activity of the tannins: their ability to interact with and precipitate proteins, including the proteins found in animal skin. The term "tannin" comes from the ancient Celtic word for oak, a topical source for tannins for leather making.

Bate-Smith defined tannins as "water-soluble phenolic compounds having molecular weights between 500 and 3000 giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and other proteins".

Vegetable tannins, nowadays often called plant polyphenols in order to provide a more adequate description of their heterogeneous chemical structures and diverse reactions (Haslam, 1989, 1998), have accompanied human life since its beginnings. As common and unavoidable components of food and beverages of plant origin, they attributed to their taste and palatability by their more or less pronounced astringency. Vegetable tannins were early recognized also as valuable chemicals for manifold technical processes. Plant tannins are also used in the production of dyes and inks or as versatile medical in traditional folk medicine, particularly in East Asia (Haslam et
The antioxidant, antimicrobial, antiviral and antitumor characteristics, as well as many other medically important properties of plant tannins, are currently intensively investigated worldwide in many laboratories (Gross et al., 1999). Over the past decades, enormous progress has been achieved in elucidating the complex structures of different plant tannins, which provided an excellent basis for subsequent studies on the biosynthesis of these compounds. Plant tannins are commonly divided into two subclasses: (i) condensed tannins (syn. proanthocyanidins) which are of flavonoid origin and (ii) hydrolyzable tannins that can be described as esters of gallic acid with a polyol (typically β-D-glucose).

### 3.8.2 Hydrolyzable Tannin

Hydrolyzable tannins are derivatives of gallic acid (3, 4, 5-trihydroxyl benzoic acid), ellagic acid and related acids. Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively cross linked to yield more complex hydrolysable tannins (Haslam, 1989). Early work on hydrolysable tannins included structure elucidation of the simple gallotannins, characterization and classification of complex hydrolysable tannins has provided useful insights into likely biosynthetic routes for the complex hydrolysable tannins (Okuda et al., 1995; Feldman et al., 2000).

The simplest member of this group, 1-O-galloyl-β-D-glucopyranose (β-glucogallin), has been identified more than a century ago as a natural product. Increasing substitution of this monoester leads to the fully galloylated glucose derivative, 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose. The various mono to penta substituted esters is often classified as simple galloylglucoses to allow their discrimination from complex galloylglucoses or gallotannins proper. This nomenclature, however, neglects the fact that even tetra and particularly pentagalloylglucose display pronounced tanning potentials. Complex gallotannins, in contrast, are the result of further galloylation reactions at the pentagalloylglucose core by which high-molecular metabolites are formed that can contain up to 10, and occasionally even more, galloyl residues. A typical representative of this group, the hexagalloylglucose (2-O-digalloyl-1,3,4,6-tetra-O-galloyl-β-D-glucopyranose) is depicted in Figure 3.16. The existence of one or more depsidic meta-digalloyl moieties is characteristic of these gallotannins.
Alternatively, pentagalloylglucose can be subjected to oxidation reactions that form linkages between suitably orientated galloyl residues to yield 3, 4, 5, 30, 40, 50-hexahydroxydiphenoyl (HHDP) moieties, thus giving rise to the second subclass of hydrolysable tannins known as ellagitannins (tellimagrandin II in Figure. 3.16). Upon hydrolysis, bound HHDP residues are liberated as free hexahydroxydiphenic acid which undergoes spontaneous conversion to the dilactone, ellagic acid. Ellagic acid is usually conjugated with a glycoside moiety (glucose, arabinose, xilose, etc.) or even more commonly, forms part of polymeric molecules called ellagitannins (Figure. 3.16) (Clifford and Scalbert, 2000). Ellagitannins are included within the so-called hydrolysable tannins that can be hydrolyzed producing ellagitannins via spontaneous lactonisation of hexahydroxydiphenic acid. A host of dimeric and oligomeric ellagitannins is produced by this means; already more than 10 years ago, the existence of more than 150 ellagitannin dimers, trimers and tetramers have been reported (Okuda et al., 1993). The occurrence of ellagitannins has been reported, among others, in walnuts, pomegranates (fruit and juice), persimmon, oak-aged wines (leakage of ellagitannins from oak barrel to wine), strawberries, raspberries, blackberries (and their derivatives such as juices, jams and jellies), peach, plum, muscadine grape and wine, etc. (Clifford and Scalbert, 2000).

In general, the gallotannin subclass are ‘simple esters’ are extended by attachment of additional galloyl residues to the phenolic galloyl-OH groups to yield meta depsidic side-chains of variable length. The ellagitannin subclass, in contrast, is characterized by oxidative linkage of spatially adjacent galloyl residues of the core unit with the formation of hexahydroxydiphenoyl bridges (Jorg and George, 1997).
Gallotannin, a subclass of hydrolyzable plant tannins, are receiving increasing attention as interesting chemotherapeutics, a function that emerged from ethnopharmacological Traditions. Antioxidant and radical scavenging activities have been investigated in relation to tumor prevention (Okuda, 1993); other potential applications regard vasodilatory effects (Goto et al., 1996) or use as diuretic and ailments of bladder and urinary tract (Haslam, 1996). As mentioned above the gallotannins are simplest hydrolysable tannins, and are simple polygalloyl esters of glucose. The prototypical gallotannin is pentagalloyl glucose (β-1,2,3,4,6-Pentagalloyl-O-D-Glucopyranose). Pentagalloyl glucose, or PGG, has five identical ester linkages that involve aliphatic hydroxyl groups of the core sugar. The alpha anomer is not common in nature. Like all of the gallotannins, PGG has many isomers. The
molecular weights of all the isomers of PGG are the same (940 g/mol), but chemical properties such as susceptibility to hydrolysis and chromatographic behavior; and biochemical properties such as ability to precipitate protein are structure-dependent.

3.8.3.1 Occurrence of galiotannins

A series of enzyme studies with cell-free extracts from *Quercus Robur*, *Q. rubra* and *Rhus typhina* has been reported to contain principle chemical constituent as galiotannins. Simple gallotannin with up to 12 esterified galloyl groups and a core glucose are routinely found in tannin from sumac (*Rhus Semialata*) galls (Chinese galiotannin); Aleppo oak (*Quercus infectoris*) galls (Turkish galiotannin); sumac (*R. coriaria, R. Typhina*) leaves (sumac galiotannin). Although commercially these galiotannins can also be prepared.

3.8.3.2 Biosynthesis of galiotannins

Gallic acid is a common precursor of gallotannin, and the main steps of its transformation via 1-O-galloylglucose into a wide range of more complex galloylglucoses (Gross, 1992, 1999; Grundhofer *et al.*, 2001; Salminen *et al.*, 2001). The mechanism of gallic acid synthesis in higher plants, on the other hand, is still an open question. Three alternative pathways have been proposed for the formation of gallic acid: (a) by α-oxidation of 3,4,5-trihydroxycinnamic acid, (b) by hydroxylation of 3,4-dihydroxycinnamic (protocatechuic) acid, and (c) by direct dehydration of 3-dehydroshikimic acid, an intermediate compound of the shikimate pathway (Gross, 1992; 1999; Werner *et al.*, 1999). The existence of pathway (a) or (b) is less likely, since the postulated precursors 3,4,5-trihydroxycinnamic acid and protocatechuic acid have been found less frequently (Ossipov *et al.*, 1995; 1996). In fact, 3,4,5-trihydroxycinnamic acid has not been encountered in higher plants (Gross, 1992; 1999; Werner *et al.*, 1999). Thus, the direct aromatization of dehydroshikimic acid (pathway c) is considered to represent the most probable mechanism of gallic acid synthesis possibly in higher plants. The direct synthesis of gallic acid from dehydroshikimic acid is supported mainly by results of experiments with glyphosate (N-[phosphonomethyl]-glycine). This herbicide blocks the shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase, and thus causes a reduction in the synthesis of aromatic amino acids and phenylpropanoids. In contrast, the synthesis
and accumulation of shikimic acid, gallic acid and gallotannins are activated (Amrhein et al., 1980; Becerril et al., 1989; Gross, 1992; Rausch and Gross, 1996). Gallic acid is formed from an intermediate compound of the upstream reactions of the shikimate pathway, most probably from 3-dehydroshikimic acid, and that the reaction is catalysed by a specific dehydrogenase (Gross, 1992; 1999; Werner et al., 1999).

3.8.3.3 Accumulation and storage of gallotannin

Gallotannins are regularly characterized by substantial accumulations of particular metabolites in certain tissues. Examples are legion; the fresh leaves of *Rhus typhina* contain 12–15% of a heptato-octagalloyl glucose derivative (syn. tannic acid) based upon the further galloylation of the key metabolite β-1, 2, 3, 4, 5-penta-O-galloyl-D-glucose; the young leaves of the tea plant (*Camellia sinensis*) contains 20–25% of the phenols (−)-epicatechin and (−)-epigallocatechin and their galloyl ester derivatives (Lunder, 1988).

3.8.3.4 Induction and regulation of gallotannin

Although Gross and his group (Gross, 1999; Haslam, 1998) have made substantial progress towards an understanding of the mechanisms involved in the formation of the pivotal metabolite β-1, 2, 3, 4, 6-penta-O-galloyl-D-glucose and the various gallotannins from glucose and gallic acid, so far as induction and regulation of gallic acid metabolism are concerned large and significant gaps in our knowledge remain. Indeed, they begin with the biosynthesis of gallic acid itself, direct dehydrogenation of an intermediate in the shikimate pathway and retention of the oxygen atoms of the alicyclic precursor (Conn and Swain, 1961; Knowles et al., 1961; Dewick and Haslam, 1969; Cornthwaite and Haslam, 1965; Werner et al., 1997). However, this places gallic acid in a potentially unique position when compared to the majority of other plant phenols which more generally derive from end-products of the pathway and the phenolic groups are derived by direct oxygen insertion into the aromatic nucleus.
3.8.3.5 Biological activities of gallotannins

Tannins have diverse effects on biological systems because they are potential metal ion chelators, protein precipitating agents and biological antioxidants. Because tannins can play such varied biological roles and because of the enormous structural variation among tannins, it has been difficult to develop models which allow accurate prediction of the effects of tannins in any system. An important goal of future is on the biological activities of tannins and the development of structure activity relationships so that biological activities can be predicted.

a. Antimicrobial activity

The antimicrobial effects of tannins have been widely recognized. Kakiuchi and co-workers (Kakiuchi et al., 1986) have studied several traditional Chinese medicines for antibacterial activity against Streptococcus mutans, a primary cariogenic bacterium. Gallotannin-rich extracts inhibited the adherence of Streptococcus mutans to smooth surfaces (Buryne et al., 1999)

b. Gallotannins as metal ion chelators

Phenolics can affect the biological availability or activity of metal ions by chelating the metal (McDonald et al., 1996). Chelation requires appropriate patterns of substitution and a pH above the pKa of the phenolic group. Bacterial siderophores with multiple phenolic groups and very high affinities for essential metals such iron have been characterized (Harris et al., 1979). The similarity between siderophores ortho-dihydroxy substitution pattern on condensed and hydrolyzable tannin suggests that tannins may also have very high affinities for metals.

c. Gallotannins as antioxidant

Although dietary tannins are often perceived as detrimental because of their potential to affect protein digestibility or metal ion availability, it is also possible that tannins are beneficial. It is likely, based on our knowledge of tannin chemistry, that tannins are biological antioxidants.
Antioxidants are widely believed to be an important line of defense against oxidative damage, which has been implicated in a range of diseases including cancer, cardiovascular disease, arthritis and ageing (Ckchrer, 1993). Biological antioxidants are generally divided into three groups: enzymes, such as superoxide dismutase; inhibitors of radical formation, such as Fenton reaction inhibitors; and free radical quenching agents, such as alpha-tocopherol (Vitamin E). Phenolics are good candidates as antioxidants because of their favorable redox potentials and the relative stability of the peroxyl-radical (Simic and Jovanovic, 1994).

Gallotannin was reported to produce antioxidant activity by preventing reactive oxygen species-mediated damage. Gallotannins inhibited the peroxyl-radical induced lipid peroxidation of L-α-phosphatidylcholine liposomes dose-dependently and prevented the bovine serum albumin from peroxyl-induced oxidative damage. Gallotannins also inhibited copper (II)-1, 10-phenanthroline complex-induced DNA oxidative damage (Zhao et al., 2005). In various in vivo and in vitro animal model oxygen free radical scavenging activity of different forms of gallates, such as gallyl esters, methyl gallate, propyl gallate, gallocatechin, gallotannins, has been documented (Buttemeyer, 2003; Ajay 2003; Calvin and Mattill et al., 1942).

d. Gallotannin in protein digestibility and precipitation

Although high levels of dietary tannin can interfere with protein utilization (Salunkhe et al., 1990) and perhaps to humans (Jankun et al., 1997), and some mammals have developed mechanisms for accommodating even rather high levels of dietary tannins (McArthur et al., 1991). Reports of tannin toxicity are generally linked to ingestion of large amounts of tannin or introduction by routes other than oral ingestion. Chemical modification of the tannin, which may occur during food preparation or cooking, may increase or decrease the toxicity of the tannin to certain animals.

Gallotannins interact with dietary protein to form indigestible protein-tannin complexes and also inactivate the enzymes (Kumar and Singh, 1984). Therefore the digestion of fiber and proteins is depressed in the ruminants (Van Soest and McDowell 1987).
e. Antihypertensive effects of gallotannins

In vivo inhibitory effect of the gallotannins on angiotensin I-induced blood pressure elevation in spontaneously hypertensive rats showed a strong dose-dependent hypotensive effect (Ju-Chi et al., 2003).

f. Inhibition of Tumor Necrosis Factor-α Production

Gallotannin analogues inhibit TNF-α secretion from lipopolysaccharide-stimulated human peripheral blood mononuclear cells (Feldman, et al., 2002).

g. Antidiabetic activity

Gallotannins from Lagerstroemia speciosa (banaba) are reported to produce antidiabetic activity in in vivo model of diabetic db/db mice and obese ob/ob mice and in invitro. They also produce glucose transport stimulatory activity in 3T3-L1 adipocytes and adipocyte differentiation inhibitory activity in preadipocytes (Liu et al., 2001). Gallotannin from Punica granatum are reported to enhance cardiac PPAR-γ mRNA expression and restore the down-regulated cardiac glucose transporter (GLUT)-4 in Zucker diabetic fatty rats (Huanga et al., 2005). They are reported to reduce blood glucose level not only in experimental animal but also in diabetic patients (Najim et al 2004; Gin et al., 1999).

h. Antihyperlipidemic activity

Gallotannins increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipogenesis and produced increased PPAR-γ dependent mRNA expression and increased activity of lipoprotein lipase in human THP-1-differentiated macrophage cells in invitro studies in Punica granatum flower extract (Huanga et al., 2005).

i. Cardioprotective effect of gallotannin

Epigallocatechin-3-gallate (EGCG) is a prominent gallotannin present in green tea. Epidemiological evidence suggests that tea consumption have a strong effect on cardiovascular disease (Duffy et al., 2001; Rajesh et al., 2004).
3.9 Phytochemistry and pharmacology of fruit of *E. officinalis*

Family: Euphorbiaceae

Latin names: *Phyllanthus emblica* Linn., *Emblica officinalis* Gaertn.

The origin of the name is from Sanskrit Amlaki. *E. officinalis* known as Amla in Hindi, and Yeowkan in Chinese, Emblic myrobalam and Indian gooseberry in English, and Phylontha emblic in French. Amla is one of the important herbal drugs used in Unani and Ayurvedic systems of medicine. It is used both as a medicine and as a tonic to build up lost vitality and vigor. In Unani medicine, it is described as a tonic for heart and brain. The fruits of *E. officinalis* are used in many medicinal preparations of Ayurvedic and Unani systems of medicine (Kritikar and Basu, 1933). According to the two main classic texts on Ayurved, Charak Samhita and Sushrut Samhita, Amalaki is regarded as “the best among rejuvenative herbs”, “useful in relieving cough and skin disease”, and “the best among the sour fruits”. According to Charaka, *E. officinalis* fruits are useful in hemorrhage, diarrhea and dysentery and acts as anabolic, antibacterial and resistance building. Many polyherbal formulations like Trifala, Cogent db, Diasulin contains *E. officinalis* as one of the ingredient (Naik *et al.*, 2006; Saravanan and Pari, 2005; Pari and Saravanan, 2002).

Figure 3.17: Fresh fruits of *E. officinalis*
3.9.1 Description

It is a tree of small or moderate size with a greenish-grey bark and greenish-yellow flowers, formed in axillary clusters. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex, subsessile, closely set along branchlets, light green and resembling pinnate leaves. The flowers are greenish yellow, borne in axillary fascicles, giving way to a globose fruit (Warrier et al., 1998; Kirtikar and Basu, 1933). The tender fruits are green, depressed, globose or oblate, indented at the base, smooth, fleshy and shining. The nearly stem less fruits are obscurely 6-lobed splitting into three segments. Fruits are about 1.5-2.5 cm in diameter with six softly defines ridges and six seeds and green at first, become whitish or a dull, greenish-yellow or more rarely, brick-red as it matures. They are hard and unyielding to the touch. The skin is thin, translucent and adherent to the very crisp, juicy, concolorous flesh. They average 5 to 6 g in weight and 4 to 5ml in volume. Ripe fruits are astringent, extremely acidic and some are distinctly bitter. They are capsular (drupaceous) berries with a fleshy exocarp. The edible part of the fruit is the mesocarp and the endocarp forms the hard stone which encages the seeds which is tightly embedded in the center of the flesh is a slightly hexagonal stone containing 6 small trigonous seeds, (each of the 3 segments usually contains 2 seeds). Each is about 4-5 mm long, 2-3 mm wide, weighing 572 mg and 590 microliters in volume (Nadkami and Nadkami, 1999).

3.9.2 Geographical distribution

It grows in tropical and subtropical parts of China, India, Indonesia, and on the Malay Peninsula (Perianayagam et al., 2005) and indigenous to tropical India and Southeast Asia (Barthakur and Arnold, 1991). In India it growing in the Deccan, the sea-coast districts and Kashmir (Nadkarni and Nadkarni, 1999). It is common all over tropical and sub-tropical India and also found in Burma, it is abundant in deciduous forests of Madhya Pradesh.
3.9.3 Chemical constituents and phytochemical investigations

Zhang and coworkers have reported that fruit juice of *E. officinalis* contains phenolic constituents like gallic acid, L-malic acid 2-O-gallate, mucic acid 2-O-gallate, corilagin chebulagic acid, putrajivain A, elacocarpusin, mucic acid,1-O-galloyl-β-D-glucose, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate, mucic acid 2-O-gallate, mucic acid 1,4-lactone 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 3-O-gallate, mucic acid 1,4-lactone 3,5-di-O-gallate (Zhang et al., 2001; Zhang et al., 2004)(Figure 3.18).

Ghosal *et al.,* 1996 have reported that fresh pericarp of *E. officinalis* contain higher amount of hydrolysable tannins like emblicanin A and B, punigluconin, pedunculagin. The structure have been established by spectroscopic analysis and chemical transformation (Figure 3.19). Kumaran and Karunakaran (2006) have performed an activity-directed fractionation and purification process to identify phytochemicals present in *E. officinalis*. They have identified gallic acid, methyl gallate, corilagin, furosin and geraniin in *E. officinalis* by chromatographic and spectroscopic methods (Figure 3.20).

Phytochemical investigations reveled that *E. officinalis* contains higher amount of flavanoid like quercetin (Anila and Vijayalakshmi, 2003; Gulati *et al.,* 1995). Fruits of *E. officinalis* were analyzed for their alkaloidal content. Alkaloids like phyllantine and phyllantidine were confirmed by chromatography and IR spectral studies. (Khanna and Bansal, 1975). Other classes of compounds elaborated by *E. officinalis* are flavanoids (e.g. quercetin, rutin, kaempferol-3-O-glucose), carbohydrate(e.g. galactaric acid) (Sukh, 2006). It has been reported by many workers that fruits of *E. officinalis* contains higher amount of Vitamin C. The fruit is a very rich source of vitamin C (Scartezzini *et al.,* 2006; Khopde *et al.,* 2001; Nisha *et al.,* 2004), but this is probably not the case. It was proposed that superior effect of the mistaken"vitamin C" component is actually the more stable and potent anti-oxidant effect of the tannins that is having structural similarity with vitamin C (Ghosal *et al.,* 1996).

The fruits of *E. officinalis* also contained considerably higher concentrations of most minerals, protein and amino acids like Glutamic acid, proline, aspartic acid, alanine, cystine and lysine (Barthakur and Arnold, 1991).
1-malic acid 2-O-gallate  
(G=gallyl)
Mucic acid 2-O-gallate  
(G=gallyl)
1-O-galloyl-β-D-glucose  
(R=H, G=gallyl)

Corilagin  
(R₁=R₂=H)
Chebulagic acid  
(R₁=R₂=che)
Elaeocarpusin Putranjivain A  
(R₁=R₂=ela) (R₁=R₂=put)

Mucic acid 6-methyl ester 2-O-gallate (R₁=H, R₂=CH₃)  
Mucic acid 1-methyl ester 2-O-gallate (R₁=CH₃, R₂=H)  
Mucic acid 2-O-gallate (R₁=R₂=H)  
Mucic acid 1,4-lactone 2-O-gallate (R₁=gallyl, 
R₂=R₃=R₄=H)  
Mucic acid 1,4-lactone 6-methyl ester 2-O-gallate  
(R₁=gallyl, R₂=R₃=H, R₄=CH₃)  
Mucic acid 1,4-lactone 3-O-gallate  
(R₁=R₂=R₃=H, R₄=gallyl)  
Mucic acid 1,4-lactone 3,5-di-O-gallate  
(R₁=R₄=H, R₂=R₃=gallyl)

Mucic acid 1,4-lactone 5-O-gallate (R=H)  
Mucic acid 1,4-lactone 6-methyl ester 5-O-gallate (R=CH₃)

Figure 3.18: Structures of phenolic compounds from fruit juice of *E. officinalis*  
(Zhang et al., 2001)
3.9.4 Pharmacological studies

3.9.4.1 Antioxidant activity

Ghosal et al., (1996) have established by comprehensive chromatographic, spectroscopic and crucial chemical analysis of fresh juice and extractive that the antioxidant effect of *E. officinalis* is not due to its rich vitamin C content but the activity is located in the low molecular weight hydrolysable tannins. Tannins like emblicanin-A, emblicanin-B, punigluconin, pedunculagin have been found to provide protection against oxygen radical induced haemolysis of rat peripheral blood erythrocytes. The mechanism of action of antioxidant activity has been suggested to be due to recycling of sugar reductone moiety and conversion of the polyphenol in to medium and high molecular weight tannins.

Antioxidant activity of tannoid principle of *E. officinalis* was investigated on the basis of their effects on rat brain frontal cortical and striatal concentration of oxidative free
radical scavenging enzymes like superoxide dismutase, catalase, glutathione peroxidase, lipid peroxidation in terms of thiobarbituric acid reactive products (Bhattacharya et al., 2000).

Nitric Oxide (NO) radical scavenging phenolic principles were quantitatively analyzed from E. officinalis by in vitro method. It was found that geraniin showed highest NO scavenging activity among the isolated compounds from E. officinalis (Kumaran and Karunakaran, 2006).

Aqueous extract of E. officinalis was found to be a potent inhibitor of lipid peroxide formation and scavenger of hydroxyl and superoxide radicals in vitro (Jose and Kuttan, 1995; Naik et al., 2005).

The antioxidant activity of free and bound phenolics of E. officinalis turmeric was investigated by Kumar et al., 2006. Higher level of antioxidant activity in E. officinalis has been attributed to the phenolic content (12.9%, w/w) in them. Gallic acid and tannic acid were identified as the major antioxidant components in phenolic fractions of E. officinalis.

Aqueous extract of E. officinalis is able to inhibit γ-radiation-induced lipid peroxidation (LPO) in rat liver microsomes and superoxide dismutase (SOD) damage in rat liver mitochondria (Khopde et al., 2001).

The antioxidant properties of E. officinalis extracts and their effects on the oxidative stress in streptozotocin-induced diabetes were examined in rats. E. officinalis showed strong inhibition of the production of advanced glycosylated end products which is a glycosylated protein that is an indicator of oxidative stress. Furthermore, thiobarbituric acid-reactive substances levels were significantly reduced with E. officinalis, indicating a reduction in lipid peroxidation. In addition, the decreased albumin and adiponectin levels in the diabetic rats were significantly improved with E. officinalis (Rao et al., 2005).

3.9.4.2 Prevention of hyperthyroidism

The ethanolic extract from the fruits of E. officinalis was investigated to evaluate its possible ameliorating effects, on the L-thyroxine (L-T4) induced hyperthyroidism in
mice. While an increase in serum T3 (triiodothyronine) and T4 (thyroxine) concentrations, and in a thyroid dependent parameter, hepatic glucose 6-phosphatase activity was observed in L-T4 (Panda et al., 2003).

3.9.4.3 Gastroprotective

An ethanolic extract of *E. officinalis* was examined for its antisecretory and antiulcer activities employing different experimental models in rats, including pylorus ligation Shay rats, indomethacin, hypothermic restraint stress-induced gastric ulcer and necrotizing agents (Al-Rehaily et al., 2002).

3.9.4.4 Immunomodulating activity

The fruits extracts of *E. officinalis* has been reported to have strong immunomodulatory properties. This immunomodulatory property was evaluated using chromium (VI) as an immunosuppressive agent. It also inhibited apoptosis and DNA fragmentation and relieved the immunosuppressive effects of Cr on lymphocyte proliferation and even restored the IL-2 and g-IFN production considerably (Sai Ram et al., 2002; Ganju et al., 2003).

3.9.4.5 Hepatoprotective activity

Hepatoprotective activity of *E. officinalis* fruit extracts were studied using carbon tetrachloride induced liver injury model in rats. Extract was found to reduce elevated levels of collagen-hydroxyproline significantly, indicating that the extract could inhibit the induction of fibrosis in rats (Jose and Kuttan, 2003; Girish et al., 2004; Jeena et al., 1999).

3.9.4.6 Prevention of cataract

*E. officinalis* is widely used against many chronic ailments including diabetic cataract. Aqueous extract of *E. officinalis* and its major constituent tannins produced inhibition against rat lens, purified recombinant human aldose reductase and sugar-induced osmotic changes (Suryanarayana et al., 2004).
3.9.4.7 Antidiarrheal

The methanol extract of *E. officinalis* showed a significant inhibitory effect on castor oil and magnesium sulfate induced diarrhea and reduction in gastrointestinal motility in charcoal meal tests in rats. It also significantly inhibited PGE2-induced enteropooling in rat (Perianayagam *et al.*, 2005; Vijayarajkumar and Vijayakumar, 2005). *E. officinalis* is used medicinally for the treatment of diarrhea. A fruit decoction is mixed with milk and given by the natives in cases of dysentery. (Nadkarni and Nadkarni, 1999).

3.9.4.8 Antitussive activity

The antitussive activity of *E. officinalis* was tested in conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial mucous areas of airways. Antitussive activity of *E. officinalis* is due to antiphlogistic, antispasmodic, antioxidant and mucus secretary activity in the airways (Nosal'ova *et al.*, 2003).

3.9.4.9 Anti-pyretic and analgesic activity

Extracts of *E. officinalis* fruits showed potent anti-pyretic and analgesic activity in several experimental models like brewer's yeast induced hyperthermia in rats, tail-immersion test and acetic acid-induced writhing response in mice (Perianayagam *et al.*, 2004). The fresh fruit is refrigerant, infusion of fruit is a good drink in fevers (Nadkarni and Nadkarni, 1999). There is a compound powder composed of equal parts of the emblica seed, chitrak root, chebulic myrobalan, pipili and saindhava also used in fever. A paste of the fruit is a useful application to the forehead in cases of cephalalgia (headache). The name “ItrifaP” of Unani medicine is the same as “Triphala” in the Ayurvedic system and represents a group of preparations used for care of headache.

3.9.4.10 Prevention of atherosclerosis and hyperlipidemia

*E. officinalis* may be effective for hypercholesterolemia and prevention of atherosclerosis. Fresh juice and ethyl acetate extract of *E. officinalis* exhibited more potent serum lipid lowering effect. Serum cholesterol, TG, phospholipid and LDL
levels were significantly decreased by the administration of *E. officinalis* (Kim et al., 2005; Thakur et al., 1988; Mathur et al., 1996; Mishra et al., 1981).

### 3.9.4.11 Antimicrobial activity

Alcoholic extracts of *E. officinalis* was found to show potential antibacterial activity against one or more test pathogens (Ahmad et al., 1998).

### 3.9.4.12 Antitumour

*E. officinalis* extract was found to inhibit cell cycle regulating enzymes cdc 25 phosphatase in a dose dependent manner that needed for inhibition of cdc2 kinase. The results suggest that antitumour activity of *E. officinalis* may partially be due to its interaction with cell cycle regulation (Jose et al., 2001). *E. officinalis* also induces apoptosis in Dalton’s Lymphoma Ascites and CeHa cell lines (Rajeshkumar et al., 2003; Sharma et al., 2000).

### 3.9.4.13 Adaptogenic

*E. officinalis* induces genotypic adaptation appeared to depend on the ability of target tissues to synthesize prostaglandins (Rege et al., 1999).

### 3.9.4.14 Antidiabetic activity

Oral administration of extracts of *E. officinalis* reduced the blood sugar level in normal and in alloxan induced diabetic rats (Sabu and Kuttan, 2002; Tripathi et al., 1979). Infusion of the fruit seeds are used in the treatment of diabetes mellitus (Nadkarni and Nadkarni, 1999).

### 3.9.4.15 Protection against ischemia-reperfusion induced oxidative stress

The tannoid principles of the fruits of *E. officinalis* have been reported to exhibit antioxidant activity in against ischemia reperfusion induced oxidative stress in rat heart. It confirms the antioxidant effect of *E. officinalis* and indicates that the fruits of the plant may have a cardioprotective effect (Bhattacharya et al., 2002; Rajak et al., 2004).
3.9.4.16 Antiproliferative activity

Phenolic compounds from the fruit juice showed stronger antiproliferative activities against MK-1 (human gastric adenocarcinoma), HeLa (human uterine carcinoma), and B16F10 (murine melanoma) cells (Zhang et al., 2004). *E. officinalis* also showed antiproliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines (Lambertini et al., 2003).

3.9.4.17 Antulcerogenic

The ulcer protective potential of methanolic extract of *E. officinalis* was assessed in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and pyloric ligation and healing effect in chronic gastric ulcers induced by acetic acid in rats (Sairam et al., 2002).

3.9.4.18 Antihypertensive effect

Acetone extract of shade dried leaf of *officinalis* shows weak angiotensin-converting enzyme inhibition action at concentration used (Nayman et al., 1998). It is also reported that ethanolic extract of dried fruit gives hypotensive activity in dogs (Dhar et al., 1968; Ishaq et al., 2005). Effect of aqueous extract of dried fruits of *E. officinalis* on the mean arterial blood pressure (MABP), heart rate and respiratory rate (RR) of the anaesthetized male dogs when given alone and in combination with various agonists as well as antagonists was studied. Aqueous extract showed a biphasic response on MABP with a slight initial increase of short duration, followed by a prominent decrease of longer duration. An increase in the rate and depth of respiration was also seen. Heart rate was decreased. Response due to carotid occlusion was not altered but the response due to vagal stimulation was enhanced by aqueous extract. Extract also potentiated the effects of histamine and acetylcholine. Initial increase in MABP was not blocked by prazocin, neither was the subsequent decrease blocked by propranolol. However both pheniramine and atropine reduced the magnitude of decrease in MABP due to aqueous extract to some extent. Observations suggest a synergistic cholinergic as well as histaminergic effect of *E. officinalis* aqueous extract on MABP and HR of anaesthetized dogs revealing its hypotensive potential (Ishaq et al., 2005).
3.9.4.19 Anti-HIV activity

The fruit of *E. officinalis* was shown to contain a number of compounds with potent inhibitory activity against human immunodeficiency virus (HIV) reverse transcriptase (El-Mekkawy *et al.*, 1995). Because reverse transcriptase plays a key role in the replication of retroviruses, this enzyme is an important potential target for the development of therapeutic agents for the treatment of HIV/AIDS. The most potent compound from *E. officinalis* in terms of inhibition of reverse transcriptase was an ellagitannin, putranjivain A (IC50 = 3.9 mcM). Other isolated compounds with potent inhibitory activity included several phenolic compounds derived from gallic acid and two flavonoid glycosides.

3.9.4.20 CNS effect

Extract of *E. officinalis* improved learning process in mice (Kulkarni and Verma, 1992). Extract mixture decreased latency in passive avoidance paradigm and number of mistakes, these being measured by time to find shock-free zone and number of stepping-downs from this zone into shock grid. The tannoid principles of *E. officinalis* exerted a prophylactic effect against neuroleptic-induced tardive dyskinesia which is likely to be due to its earlier reported antioxidant effects in rat brain areas, including striatum (Bhattachrya *et al.*, 2000).

3.9.4.21 Antituberculosis action

The report showed the hepatoprotective property of a 50% hydroalcoholic extract of the fruits of *E. officinalis* against antituberculosis drugs-induced hepatic injury. *In vitro* studies were done on suspension cultures of rat hepatocytes while sub-acute studies were carried out in rats.

3.9.5 Therapeutic uses of *E. officinalis*

*E. officinalis* is among the most important medicinal plants in the Ayurvedic Meteria Medica. It has been used a single substance or as a valuable ingredient of various medicines or formulations in various disorders.
3.9.5.1 Appetite inducer

The green fruits are made into pickles and preserves to stimulate the appetite.

3.9.5.2 Aphrodisiac activity

_E. officinalis_ is believed to increase ojas, and is considered to be one of the strongest rejuvenative herbs in Ayurvedic medicine. It is the primary ingredient used in one of the renowned Ayurvedic herbal formulation, called Chayavanprasha which is used as a sexual rejuvenative tonic (Puri, 1971).

3.9.5.3 Boils and spots

The pericarp of the fruit is often used in decoctions along with other ingredients like cow ghee applied externally on boils.

3.9.5.4 Constipation

According to Nadkami fresh fruit is extensively used as a laxative in India (Nadkami and Nadkami, 1999). Fruit along with those of _Terminalia bellirica_ and _Terminalia chebula_ are the constituents of "Triphala" which are also used as a laxative.

3.9.5.5 Diuretic

The fresh fruit is used as a diuretic. A paste of the fruit alone or in combination with _Nelumbium speciosum_ (the Egyptian Lotus), saffron and rose water is a useful application over the pubic region in irritability of the bladder and retention of urine (Nadkami and Nadkami, 1999). Aqueous juice prepared from the fresh fruit with honey is a favored cooling drink which has a diuretic effect.

3.9.5.6 Hair growth

Indian gooseberry is an accepted hair tonic in traditional recipes for enriching hair growth and also pigmentation. The oil is said to be excellent for preventing hair graying. A fixed oil is obtained from the berries that are used to strengthen and promote the growth of hair. The dried fruits have a good effect on hair hygiene and
have long been respected as an ingredient of shampoo and hair oil (Thakur et al., 1988).

3.9.5.7 Nose bleed

The seed are fried in ghee and ground in conjee (the liquid from boiled rice) is applied to the forehead to stop bleeding from the nose.

3.9.5.8 Indigestion

Fruit is carminative and stomachic.

3.9.5.9 Mouth ulcers

A decoction of fruit and leaves is used as a chemical free bactericidal mouthwash. Another remedy suggests *E. officinalis* rubbed with honey is used in aphthous stomatitis (an inflammation of the mouth (Treadway and Linda, 1994).

3.9.5.10 Nausea

*E. officinalis* powder is mixed with red sandalwood (Pterocarpus santalinum) and prepared in honey to relieve nausea and vomiting.

3.9.5.11 Purities

The seed are burnt, powdered and mixed in oil as a useful application for scabies or itch.

3.9.5.12 Respiratory problems

The fresh fruit is used in Turkeystan in inflammations of the lungs. The juice or extract of the fruit is mixed with honey and given to stop hiccough and also in painful respiration. The expressed juice of the fruit along with other ingredients is used to cure cough, hiccough, asthma and other diseases like dyspnoea (Nadkarni and Nadkarni, 1999).
3.9.5.13 Tonic

The juice of the fresh fruit when mixed with ghee is considered a good restorative tonic. *E. officinalis* seeds and gokhru (*Tribulus terrestris*) powdered and mixed with essence of gulancha (*Tinospora cordifolia*) and given early morning in ghee and sugar is an equally nutrient tonic. These rejuvenation and longevity tonics are known as the “rasayana” (Nadkarni and Nadkarni, 1999).

3.9.5.14 Vaginal complaints

A mixture of the fruit juice and sugar is used for the relief of burning in the vagina (Nadkarni and Nadkarni, 1999).

3.9.5.15 Vermifuge

*E. officinalis* fruit juice is used as a vermifuge (Nadkarni and Nadkarni, 1999).