INTRODUCTION

Diabetes Mellitus (DM) may be considered almost as an inborn error of metabolism manifesting at varying periods of life, as a result of an absolute or relative insufficiency of endogenous insulin or defective insulin action with a tendency for hyperglycemia accelerated macrovascular disease and a highly characteristic and specific microangiopathy, which may be considered as a hallmark of diabetes. Thus it is expressed both as a metabolic and vascular disease.

1.1. Classification

The two broad categories of DM are designated as type 1 and type 2 DM. Type 1 diabetes results from β-cell destruction usually leading to absolute insulin deficiency. Type 2 DM is a heterogeneous group of disorders usually characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production.

1.2 Aetiology of type 2 diabetes

Considering the aetiology of type 2 diabetes it is clear that despite being a multifactorial disease, there are two main factors which stand out distinctly in the causation of this type of diabetes.

(i) Genetic factors

(ii) Environmental factors.
Chapter I

Father (Diabetic / Diabetic Family)

Obesity
Over Nutrition
Refined Food
Lack of Physical Activities

Prediabetic State
Repeated Pregnancies
Infections
Emotional and other stress

Diabetogenic Drugs

Mother (Diabetic / Diabetic Family)

DIABETIC

Fig. 1 Evolution of the diabetic

Genetics
The majority of cases of type 2 DM are multifactorial in nature with indication of environmental and genetic factors. The nature of the genetic contribution is largely unknown but it is evident that several genes are involved. In the polygenic model, inheritance of abnormalities in individual genes would not be sufficient to cause type 2 DM directly, but would confer to an increased or decreased susceptibility. A variety of candidate susceptibility genes have been investigated, such as those of insulin, the insulin receptor, glucose transporters and glycogen synthase.

**Environmental factors**

**Lifestyle**

Epidemiological studies of type 2 DM provide evidence that overeating, especially when combined with obesity and under activity, is associated with the development of type 2 diabetes. Obesity probably acts as a diabetogenic factor in those genetically predisposed individuals to develop type 2 DM.

**Malnutrition in utero**

It is proposed that malnutrition in utero and in infancy may damage β-cell development at a critical period predisposing to type 2 DM later in life.

**1.3 Epidemiology**

The prevalence of diabetes is increasing worldwide, and current estimates indicate that worldwide about 150 million people have type 2 DM with the figures expected to double by 2025 (Zimmet et al., 2001). The Indian scenario is equally bad with rapid urbanization and industrialization leading to westernization of lifestyle patterns. The consequent growing epidemic of obesity is responsible for an increase in the incidence and prevalence of type 2 DM which represents almost 90% of all cases of diabetes (Monteiro et al., 2005). This had led to an increase in CVD (Cardiovascular disease) and its consequent morbidity and mortality.
1.4 Pathophysiology

Type 2 DM is characterized by three pathophysiologic abnormalities impaired insulin secretion, peripheral insulin resistance and excessive hepatic glucose production.

Insulin resistance

This is caused by the decreased ability of insulin to act effectively on peripheral target tissues and is a prominent feature of type 2 DM. The resistance is relative, since supernormal levels of circulating insulin will normalize the plasma glucose. Insulin dose-response curves exhibit a rightward shift, indicating reduced sensitivity and a reduced maximal response, indicating an overall decrease in maximum glucose utilization. Resistance to the action of insulin impairs glucose utilization by insulin sensitive tissues and increases hepatic glucose output, both effects contributing to the hyperglycemia of diabetes.

Increased hepatic glucose output predominantly accounts for increased glucose usage resulting in postprandial hyperglycemia. In skeletal muscle there is a greater impairment in non-oxidative glucose usage than in oxidative glucose metabolism through glycolysis. Glucose usage in insulin independent tissues is not decreased in type 2 DM.

Impaired insulin secretion

In type 2 DM insulin secretion initially increases in response to insulin resistance in order to maintain normal glucose tolerance. Initially the insulin secretory defect is mild and selectively involves glucose stimulated insulin secretion.

Eventually, the insulin secretory defect progresses to a state of grossly inadequate insulin secretion. Some endogenous insulin production continues, but the amount secreted is less than the amount secreted by normal individuals at the same plasma glucose concentration.
**Increased hepatic glucose production**

In type 2 DM insulin resistance in the liver arises from the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycemia and decreased glucose storage by the liver in the postprandial state. Increased hepatic glucose production occurs early in the course of diabetes, after the onset of insulin secretory abnormalities and insulin resistance in skeletal muscle. (Harrison's principle of internal medicine)

**Inflammatory mechanisms of type 2 diabetes**

Insulin resistance is well established as the pathophysiologic hallmark of type 2 DM. Although the exact mechanisms underlying the development of insulin resistance have not been thoroughly described, activation of inflammatory processes appears to play a significant role. The importance of inflammation is demonstrated in part by the fact that production of pro-inflammatory cytokine mediators is increased under conditions of obesity and insulin resistance. Interleukin-6 and Tumour necrosis factor-α are two such cytokines whose expression is enhanced in the plasma and adipose tissue of obese, insulin resistant subjects compared to lean subjects with normal insulin sensitivity (Keen *et al.*, 2001).

The activity of adipose tissue, particularly within the adipose fat bed, has been implicated in the pathogenesis of inflammatory activation related to insulin resistance. Circulating factors produced by adipose cells (including IL-6 and TNF-α among others) affect carbohydrate metabolism, lipid metabolism and insulin action. Additionally, a positive correlation exists between the measures of increased adiposity (i.e.,) BMI and waist to hip ratio and markers of chronic systemic inflammation including C-reactive protein (Yudkin *et al.*, 1999).
There has been a recent explosion of studies showing that important cytokines and adipokines are produced and released from fat tissue. Many of these are proinflammatory, but several proteins including adiponectin can have beneficial actions to reduce inflammation and improve endothelial function (Truj et al., 2006).

Inflammatory activation may contribute to the pathogenesis of type 2 DM in part through pancreatic β-cell dysfunction, which occurs on the setting of hyperglycemia. It is clear that β-cell dysfunction continues despite treatment of diabetes with currently available pharmacologic approaches, but thiazolidinediones appear to offer the best means to slow the decline of β-cell function (Kahn et al., 2006).

In addition to the involvement in the development of diabetes itself, inflammatory activation may also be important in diabetic complications such as atherosclerosis, diabetic nephropathy and diabetic retinopathy. The mechanisms through which inflammatory mediators contribute to these processes primarily involves macrovascular and microvascular damage.

1.5 **Clinical signs and symptoms**

- Polyuria
- Polydipsia
- Polyphagia
- Tiredness and weakness
- Loss of weight
- Slow healing of cuts and wounds
- Skin infections
- Blurred vision
- Dry or itchy skin
1.6 **Complications**

Complications are classified into acute and chronic complications. Acute complications in type 2 DM may be grouped into:

(i) **Metabolic**

- Hyperosmolar Coma (HC)
- Diabetic Ketoacidosis (DKA)
- Lactic acidosis (LA)
- Hypoglycemia (HG)

(ii) **Non-metabolic**

Here type 2 DM is causally related. This includes myocardial infarction, cerebrovascular mischief, overwhelming sepsis etc., each of which may precipitate a hyperglycemic crisis.

Chronic complications are classified into micro and macrovascular complications. Microvascular complications include:

- Retinopathy
- Nephropathy
- Neuropathy

Macrovascular complications include:

- Coronary artery disease (CAD)
- Cerebrovascular disease (CVD)
- Peripheral vascular disease (PVD)

1.7 **Diagnosis**

Diagnosis of type 2 diabetes can be made by the following tests.

- Urine testing for glucose and ketones
- Measurement of fasting and postprandial blood glucose
- Oral glucose tolerance test (OGTT)
1.8 **Management**

Three methods of treatment are available for diabetic patients.

- Diet alone
- Diet and oral hypoglycemic drugs.
- Combination therapy (Diet + oral hypoglycemic agents + insulin)

1.9 **Peroxisome proliferator activated receptors (PPARS)**

PPARS are ligand-activated transcription factors belonging to the nuclear receptor super-family, which include receptors for steroids, retinoid and thyroid hormones. (Hemberger *et al.*, 1996). After the activation of PPARs by ligand binding, they form heterodimers with the ligand-activated retinoic acid receptor (RXR).

Through its DNA binding domain this heterodimer binds to specific DNA sequences, called PPAR-responsive elements (PPRE) and induces transcriptional activation of specific genes as given in fig. 2 (Wilson *et al.*, 2000). PPARs function as regulators of glucose, lipid and protein metabolism and influence cellular proliferation, differentiation and apoptosis. They also play a role in neoplastic proliferation and inflammatory diseases (Chinetti *et al.*, 2000).

Three types of PPARs are known. They are PPAR-α, PPAR-γ and PPAR-δ. The tissue distribution of these sub types varies considerably. PPAR-δ is found in liver, intestine, kidney, heart, adipose tissue, skeletal muscle and recently in vascular cells. PPAR-δ has an important role in lipid metabolism. Its molecular targets include genes for enzymes that are important for the β-oxidation of fatty acids (Elangbam *et al.*, 2001). Synthetic ligands for this receptor sub type are fibric acid derivatives, which are used in clinical practice as lipid lowering agents. PPAR-γ is found in adipose tissue, pancreas, skeletal muscle and vasculature (Dubois *et al.*, 2000).
**Fig. 2** Mechanism of action of the peroxisome proliferator activated receptors (PPARS)

\[\alpha = \text{Ligand}; \ PPRE = \text{PPAR responsive elements}; \ RXR = \text{retinoic acid receptor}; \ TZD = \text{thiazolidinediones.}\]

**1.10 Thiazolidinediones**

The medication class of thiazolidinedione (TZD) was introduced in the late 1990s as an adjunctive therapy for DM (type 2) and related diseases. TZD structure is as below

![TZD structure](image)

Thiazolidinediones are a new class of drugs that act primarily by improving insulin sensitivity in different target tissues such as liver, skeletal muscle and adipose tissue. They are potent synthetic ligands for PPAR-\(\gamma\) activation. They have been
shown to improve glycemic control in patients with type 2 DM and appear to have favourable direct effect on other components of the insulin resistance syndrome because of the role of PPAR-γ in vascular physiology as given in Fig. 3.

![Fig. 3](image_url)  

**Fig. 3** The central role of peroxisome proliferator-activated receptor (PPAR-γ) in vascular physiology.

Thiazolidinediones are chemically and functionally unrelated to other classes of oral antihyperglycaemic agents. Two compounds in this class are currently available for clinical use, viz., *rosiglitazone*, which was approved by the US food and drug Administration (FDA) in May 1999 and *pioglitazone*, which was approved in July 1999. *Troglitazone*, the first drug of this class, was marketed in the US from March 1997 until it was withdrawn in March 2000, when the FDA decided that the risk of hepatotoxicity associated with *Troglitazone* therapy outweighed its potential benefits.

### 1.11 Chemical structure of thiazolidinediones

**Rosiglitazone**

*Rosiglitazone* (RG) is an anti-diabetic in the TZD class of drugs. It is marketed by the pharmaceutical company GlaxoSmithKline as a stand-alone drug (*Avandia*) and in combination with metformin (*Avandamet*) or with *glimepiride* (*Avandaryl*). A study involving 5,269 participants at 191 clinics in 21 countries showed that taking the drug *rosiglitazone* reduced the chance of getting type 2 diabetes by 60 per cent among those at high risk. The trial was co-ordinated by the DM Trials Unit at Oxford University and the
Canadian Cardiovascular Collaboration at McMaster University, Canada. Some reports have suggested that rosiglitazone is associated with a statistically significant risk of heart attacks, but other reports have disagreed, and the controversy has not been resolved. The molecular formula of RG is C\text{18}H\text{19}N\text{3}O\text{3}S. The molecular mass is 357.11 g/mol. The IUPAC name is 5-(4-(2-(methyl (pyridin-2-yl)amino)ethoxy)benzyl)thiazolidine-2,4-dione (Goodman and Gilmann, 2006). It is a white solid substance and the type of bonding in the structure is covalent bonding. It has a single chiral centre and exists as a racemate. It is soluble in ethanol and buffered aqueous solution (pH = 2.3).

Fig. 4 Structure of rosiglitazone
Fig. 4 Structure of *rosiglitazone*

5-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy)benzyl)thiazolidine-2,4-dione

**Pioglitazone**

The molecular formula of *pioglitazone* (PG) hydrochloride is C_{19}H_{20}N_{2}O_{3}S HCl. The molecular mass of PG is 356.44 g/mol. The IUPAC name is 5 - [4- [2-(5-ethyl pyridine - 2yl) ethoxy] Phenyl] methyl] -1, 3 - thiazolidine - 2,4-dione. (Goodman and Gilman, 2006)

It is a white crystalline powder. In the crystal structure, the molecules are linked by N-H.Cl bonds in the chain. It contains an ethyl substituent located on the fifth portion of pyridylyl ring of the TZD structure. The molecule contains an asymmetric carbon and exists as a racemic mixture. The two enantiomers of pioglitazone are interconvertible in *vivo*.

It is soluble in N,N - dimethyl formamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone, acetonitrile, methanol and insoluble in ether and water. (Pfutzner and Frost, 2006).
Fig. 5 Structure of Pioglitazone
5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)benzyl)thiophene-2,4(3H,5H)-dione
1.12. Pharmacokinetics

*Pioglitazone* is extensively metabolized by hydroxylation and oxidation, in the liver by cytochrome P450 isoforms, CYP 3A4 and CYP 2C8 into a series of active metabolites. The serum half life of *pioglitazone* is 3-7 hours and that of its metabolites is 16-24 hours. Of an administered dose, 15-30% is excreted in the urine.

*Rosiglitazone* is metabolized in the liver by cytochrome P450 isozymes, CYP 2C8 and to a lesser degree, CYP 29. It is N-demethylated, hydroxylated and then conjugated with sulphate and glucuronic acid. The metabolites are considerably less active than the active compound. The plasma half life of *rosiglitazone* is 3-4 hours. Of an administered dose, 64% is excreted in the urine and 23% in the faeces. (Cox *et al.*, 2000). The therapeutically effective dose of the glitazones are as follows:

- **Pioglitazone** - 15 to 45 mg/day
- **Rosiglitazone** - 4 to 8 mg/day

1.13 Clinical Pharmacology

1.13.1 Thiazolidinediones and glycaemic control

Several thiazolidinediones have been shown to improve insulin sensitivity by increasing glucose disposal in skeletal muscle and decreasing hepatic glucose synthase activity and glucose metabolism not only in skeletal muscle but also in adipocytes. They also decrease gluconeogenesis in cultured hepatocytes (Raman *et al.*, 2000). Stimulation of PPAR-γ normalizes glucose uptake associated with glucose transporter 4 (GLUT 4) expression and stimulates insulin receptor and activation (Shimaya *et al.*, 1998).

**Differentiation of Adipocytes**

PPAR-γ is expressed mainly in adipose tissue and is a key factor in the differentiation of adipocytes and adipogenesis (Chawla *et al.*, 1994). PPAR-γ
stimulation alters adipocyte metabolism by increasing the expression of specific adipocyte genes involved in glucose regulation; e.g., GLUT-4, lipoprotein lipase (LPL), fatty acid transporter protein, Acyl COA synthease and malic enzymes. (Vidal Puig et al., 1997).

Modulation of tissue triglyceride content

Improvement in insulin resistance is associated with a decrease in the triglyceride content of liver and skeletal muscle. Treatment with thiazolidinediones reduce the triglyceride content in liver and skeletal muscle which may be an important factor in the observed improvement in peripheral glucose disposal and decreased hepatic glucose output. In addition, thiazolidinediones also lower the triglyceride content of β-cells which is associated with an improvement of β-cell function.

Effect on Adipocyte-derived factors

Thiazolidinediones also regulate the storage and release of adipocyte derived signalling factors that affect insulin sensitivity of muscle. These factors include free fatty acids, adiponectin, TNF-α and leptin.

Free fatty acids

Fatty acids are key mediators of adipocyte derived signaling factors affecting insulin sensitivity. High levels of free fatty acids have been linked to the induction of insulin resistance, because increased free fatty acid metabolism in the liver leads to increased gluconeogenesis. There is evidence for a direct regulatory effect of fatty acids on the production of macrophage lipoprotein lipase in the vascular wall. (Michand et al., 2001).

Adiponectin

Adiponectin is adipocyte derived hormone that decreases insulin resistance by lowering the triglyceride content of muscle and liver. Thiazolidinediones can markedly enhance the expression and secretion of adiponectin in vitro and in vivo, possibly
mediated by antagonising the suppressive effect of TNF-α on the production of adiponectin. (Maeda et al., 2001).

**Tumour necrosis factor - α**

The expression of TNF-α by adipose tissue is up-regulated in obesity patients with features of insulin resistance syndrome. This cytokine decreased PPAR-γ expression, insulin receptor synthesis and activation, glucose uptake in adipose tissue skeletal muscle and liver by attenuating the expression of the glucose transporter GLUT-4 (Stephene et al., 1997)

Chronic hyperglycaemia is associated with increased TNF-α production which may be derived from adipose tissue. Thiazolidinediones restore sensitivity to insulin by downregulating adipose cytokines such as TNF-α (Fukozawa et al., 1999).

**Leptin**

Thiazolidinediones have also been implicated in the regulation of leptin expression. Administration of thiazolidinediones reduces the expression of leptin, mRNA and protein adipocytes (Devas et al., 1996).

**1.13.2 Other vascular effects of thiazolidinediones**

**Improvement of endothelial function**

Endothelial dysfunction appears to be an important feature of the insulin resistance syndrome. Thiazolidinediones bind to endothelial NO synthase (e Nos), and stimulates NO production, resulting in vasodilation. This vasodilation is impaired in insulin resistant states, which has been termed as vascular insulin resistance. Quenching of NO by decreased No or an increased inactivation of NO by reactive oxygen species (ROS) might be a major driving force for instability of atherosclerotic plaque in patients with diabetes.
Reduction of glucose and free fatty acid concentrations by thiazolidinediones will have beneficial effects since high levels of glucose and free fatty acids stimulate ROS production. Direct effects of thiazolidinediones on vascular smooth muscle cells have also been observed. Thiazolidinediones attenuate vasoconstriction as well as inhibit L-type Ca\(^{2+}\) currents in vascular smooth muscle cells (Zhang et al., 1994).

**Decreased inflammatory conditions**

Low grade inflammation plays an important role in the initiation and progression of cardiovascular diseases. Accumulation of monocyte derived lipid loaded macrophages or foam cells, smooth muscle cell proliferation and de novo formation of the atherosclerotic plaque takes place. Markers of inflammation, such as CRP, TNF-\(\alpha\), IL-6 are increased in patients with insulin resistance syndrome. Thiazolidinediones have an important role in modulating the inflammatory markers (Moore et al., 2001).

1.1.3.3 Effects on the lipid profile

**Reduction of plasma triglycerides**

The triglyceride lowering action of PPAR-\(\gamma\) activation may be the result of a reduction in fatty acid and triglyceride synthesis and consequently a decrease in the production of VLDL.

**Effects on lipoprotein metabolism**

In general there appears to be an increase in the concentration of HDL (upto 20%). The increase in HDL levels is likely to be explained by the decrease in triglyceride levels (Raskin et al., 2001). The increase in total cholesterol and LDL cholesterol is caused by the larger, buoyant LDL particles.

1.1.3.4 Lowering blood pressure

Animal and human studies have shown that thiazolidinediones decrease blood pressure associated with decreased insulin levels and improvement of endothelial
function (Grinsell et al., 2000). They may lower blood pressure by a direct vascular effect involving decreased calcium uptake into vascular cells. Alternatively, a TZD induced decrease in the activity of the renin-angiotensin system and of the sympathetic system may also play an important role in the modulation of blood pressure.

1.13.5 Additional anti-atherogenic effects

Intimal hyperplasia

The proliferation and migration of vascular smooth muscle cells play an important role in the pathogenesis and progression of atherosclerosis. Thiazolidinediones decrease the intimal-media thickness of the carotid arteries considerably by involving insulin sensitivity (Mowaed et al., 1996).

Effects on the prothrombotic state

Increased levels of the inhibitor of fibrinolysis and plasminogen activator inhibitor -1 (PAI-1) and plasma fibrinogen create a prothrombotic state. Thiazolidinediones may have favourable effects on cardiovascular events by improvement of the prothrombotic state (Kato et al., 1999).
SCOPE OF THE STUDY

It is well known that type 2 DM particularly when inadequately controlled and accompanied by other risk factors such as hypertension and dyslipidemia predisposes to a number of adverse health consequences including accelerated rates of atherosclerotic cardiovascular disease, renal insufficiency, end stage renal disease, retinopathy and peripheral neuropathy. These complications are responsible for the vast majority of diabetes-related morbidity and mortality and are largely dependent on the detrimental effects of diabetes on vasculature.

Abnormal activation of inflammatory processes is gaining recognition as a possible unifying explanation for the micro vascular and macro vascular injury that occurs in the setting of diabetes. In addition, inflammation is thought to mediate the development of insulin resistance and pancreatic β-cell dysfunction.

The present study involving the two thiazolidinediones rosiglitazone and pioglitazone may present as effective options for management of diabetic complications, which focus specifically on modulation of the immune system and allow for targeted therapy against chronic inflammatory activation. Such novel approach have the potential to be of tremendous benefit to the currently rising prevalence of type 2 DM and the projected impact of diabetic complications. The objectives of the present study include:

1) Analysis of biochemical, haematological, immunological and inflammatory parameters in type 2 diabetics.

2) Assessment of biochemical, haematological immunological and inflammatory parameters in rosiglitazone treated type 2 diabetics.

3) Evaluation of biochemical, haematological immunological and inflammatory parameters in pioglitazone treated type 2 diabetics.

4) Comparison of efficacy between rosiglitazone and pioglitazone treated type 2 diabetics.
TZDs not only reduce glycaemia but also enhance vascular function and ameliorate dyslipidaemia and inflammatory milieu of type 2 diabetes. TZDs primarily activate PPAR-\(\gamma\) receptors in adipose tissue and alter adipose metabolism and distribution. The redistribution of tissue triglyceride from visual stores reduces the level of circulating NEFA (Non-esterified fatty acids) apparently by sequestration in a less lipolytic subcutaneous compartment. (Ye et al., 2004).

TZDs reduce circulating concentrations of proinflammatory cytokines that promote insulin resistance (TNF-\(\alpha\) and IL-6). At the same time, they increase the concentrations of adiponectin which increases insulin sensitivity and anti-inflammatory properties.

The multiple effects of TZDs on adipose tissue metabolism and crosstalk of these signals with liver and skeletal muscle, as well as pancreatic beta cells and the vascular endothelium might account for the enhancement of insulin action and improvement in insulin secretion with these agents, as well as several beneficial effects on vascular function (Meriden et al., 2004).

Accumulating evidence suggest that these drugs not only significantly improve insulin sensitivity but also may reduce microalbuminuria and diabetic nephropathy in genetically obese diabetic rodents and patients with type 2 diabetes. (Yashimoto et al., 1997).

Although the renal protective action may be a result of the hypoglycemic, antihypertensive and antihyperlipidemic effects of PPAR-\(\gamma\) activation, it is also possible that these drugs exert direct effects on renal glomerular PPAR-\(\gamma\) receptors as demonstrated by many studies. (Yang et al., 1999).
In normal human islet cells PPAR-γ is highly expressed at the level of both mRNA and protein, supporting the concept of a direct influence of PPAR-γ agonists such as TZDs on the islet β cell.

The proinflammatory cytokine TNF-α is implicated in the development of insulin resistance associated with obesity in insulin sensitive peripheral cells such as those of skeletal muscle, liver and adipose tissue. TNF-α directly interferes with insulin receptor signalling by inducing phosphorylation of insulin receptor substrate-1 blocking the biological actions of insulin. (Hotamisligil et al., 1996).

It has been shown in isolated islets from Sprague-Dawley rats that TNF-α induces insulin resistance in β cells themselves, an effect which is mediated by a functional β cell insulin receptor. This insulin resistance of the β cell failure and the inability of β cells to compensate for increased insulin demand results in the development of type 2 diabetes (Kwon et al., 1999).

According to the "lipotoxicity hypothesis" β cell dysfunction in adipogenic type 2 diabetes is the result of excessive accumulation of fat in the pancreatic islets. In obese Zucker diabetic fatty (ZDF) rats, the over accumulation of fat reflects a mutation in the leptin receptor which blocks the normal triglyceride lowering action of leptin on islets. This leads to lipotoxicity through exaggerated production of NO (Skimabukuro et al., 1997).

In the same animal model, hyperglycemia was observed to occur in parallel with net β cell apoptosis. It appears that β cell proliferation initially compensates for the loss of β cells while plasma glucose is modestly elevated, but ultimately fails.

**Rosiglitazone** treatment was found to maintain β cell proliferation and to produce a 5 fold attenuation in the net rise in β cell death, preventing the loss of β cell mass (Finegood et al., 2001).
Excessive β cell apoptosis is associated with excessive accumulation of intracellular TG. It was recently shown that pioglitazone treatment in diabetic mice reduced the triglyceride content of islets by 58% suggesting that Pioglitazone prevents β cell damage by mitigating lipotoxicity (Kawasaki et al., 2002).

In isolated human pancreatic islets, expression of PPAR-γ was markedly and time-dependently reduced by exposure to progressively higher concentrations of FFA (Free fatty acids) (Lupi et al., 2004). In the same model, high concentration of FFA produced an almost 3 fold increase in rates of islet cell death, in association with significant increase in the activity of the protease enzymes-caspase 3 and caspase 9, which are key mediators of apoptosis. Incubation with rosiglitazone at a concentration of 15 µg/ml attenuated islet cell death and normalised caspase activity (Marselli et al., 2002).

In addition to enhancing insulin synthesis, TZDs have shown direct effects on β-cell function. Prigeon et al (1998) and other groups have reported that TZDs lower the ratio of proinsulin to immuno reactive insulin (IRI) in patients with type 2 diabetes.

In one multicentre, randomized double blind trial, pioglitazone (upto 45 mg once daily) was found to decrease HbA1c to ≤ 9% (Tan et al., 2004). The above data consistently revealed sustained positive effect of TZD treatment on glycaemic control as shown by reductions in HbA1C and markers for insulin sensitivity.

Fasting plasma insulin levels are frequently measured in clinical trials of diabetes as an indicator of insulin sensitivity. Decrease in FPI levels have been reported in the vast majority of trials performed with pioglitazone, mirroring a decreased secretory demand on β cells. In patients taking pioglitazone 30-45 mg / day, reductions in FPI have been in the range of 10.7 -31.8 p mol/L with monotherapy (Herz et al., 2003).
Similar to the trials evaluating pioglitazone, the majority of studies that have assessed the impact rosiglitazone on FPI have found significant reductions compared with baseline levels. The mean rosiglitazone induced reduction in FPI ranged from 6.6 to 27.2 pmol/L. (Heboritz et al., 2001).

Several studies demonstrate the beneficial effects of TZDs on recovery of improvement of β cell function as well as in one trial β cell mass. The recovery of pancreatic β cell function may be mediated by reductions in FFA and FFA metabolites within the β cell (Gastaldelli et al., 2004).

Another study found that a decrease in intramyocellular triglyceride, noted with pioglitazone was not accompanied by an increase in muscle succinate dehydrogenase activity. This suggests that beneficial effects in terms of improved insulin sensitivity, β cell function and β cell mass resulted from redistribution of fat rather than increased fat oxidation (Rosouli et al., 2004). Overall, it appears that TZDs can promote recovery of β cell function independently of the amelioration of insulin sensitivity.

Plasma FFA levels are increased and fatty acid utilization is impaired in type 2 diabetic patients (Kelley et al., 1994). TZDs have been shown to lower plasma FFA levels in type 2 diabetic patients. They have been identified as peroxisome proliferator activated receptor -γ activators. Both fat and fatty acid transport protein have been shown to be regulatable by PPAR -γ (Martin et al., 1997).

The insulin-sensitizing TZDs decrease plasma FFA levels in vivo (Patel et al., 1999). Possible mechanisms for this response could include reduced lipolysis and FFA release by adipose tissue, reduced hepatic lipogenesis or increase in FFA uptake into muscle. Rosiglitazone have been shown to increase FFA transport into 3T3-L1 adipocytes (Frohnert et al., 1999).
TZD treatment increased only the protein-mediated component of FFA uptake suggesting that TZDs upregulate transporter levels or increase transporter activity, the latter possibly through translocation to the cell surface.

TZDs have been found to increase uptake and oxidation of FFA by muscle in several animal models of insulin resistance. (Ide et al., 2000). TZDs treatment increases FFA metabolism in concert with increased expression of the lipid scavenger and transport protein FAT/CD 36. The ability of TZDs to stimulate FFA metabolism in muscle may contribute to enhanced sensitivity of insulin-stimulated glucose metabolism in the tissues of type 2 DM patients after treatment.

Experimental studies in animals and evidence from prospective and longitudinal studies in humans are consistent with an etiologic role of subclinical inflammation in the pathogenesis of type 2 DM, primarily as a mediator of obesity induced insulin resistance.

A number of studies have reported increased acute phase proteins and other non-specific markers of inflammation in type 2 DM (Leinonen et al., 2003). This is not particularly surprising, since inflammatory processes in affected tissues accompany some of the chronic complications of type 2 DM.

Obesity was found to be associated with non-specific measures of activation of the immune system such as total $\gamma$-globulin concentration (Lindsay et al., 2001), body temperature, white blood cell count and C-reactive protein. (Pratley et al., 1995; Visser et al., 1999). Associations between fibrinogen and clinical features of the metabolic syndrome as well as associations between oral temperature or white blood cell count and insulin sensitivity have also been reported (Facchini et al., 1992).
In most of the cases, the association between inflammatory markers and insulin resistance was found to be independent of adiposity. It has been suggested that inflammation is a possible pathophysiological link between obesity and insulin resistance.

Many prospective studies in diverse human populations have identified proinflammatory cytokines, acute phase proteins and several indirect markers of inflammation as predictors of type 2 diabetes. This predictive effect of inflammation on the risk of type 2 diabetes does not seem to depend on subclinical cardiovascular disease, undiagnosed diabetes at baseline or surprisingly initial degrees of insulin resistance. (Pradhan et al., 2001; Nakanishi et al., 2003).

Adiponectin has been related to insulin resistance and diabetes not only because of its cAMP-activated protein kinase effects on FFA metabolism and glucose uptake but also because of its anti-inflammatory properties. Inhibition of phagocytic activity and TNF-α production by macrophages and inhibition of the TNF-α induced expression of adhesion molecules are some of the known mechanisms by which adiponectin mediate its anti-inflammatory effects. Many studies have now suggested that human adiponectin is more closely related to insulin resistance than to obesity (Pittas et al., 2004).

Prospective and longitudinal studies have found a correlation between low adiponectin levels and a higher risk of diabetes, independent of many confounders including obesity and other inflammatory markers (Duncan et al., 2004). Krakoff et al. (2003) hypothesized that in studies in which substantial baseline differences in the degree of adiposity exist, the predictive value of inflammatory marker may be a result of their association with obesity (i.e.,) they may be acting as surrogate markers of hypoadiponectinemia and may be only indirectly associated with the development of diabetes.
Increased glucose metabolism can lead to a rise in mitochondrial production of ROS. ROS production is elevated in obesity, which causes enhanced activation of inflammatory pathways (Hin et al., 2005).

Inflammatory cytokine stimulation can also lead to induction of iNoS. Over production of nitric oxide also appears to contribute to impairment of both muscle cell insulin action and β cell function in obesity. Deletion of iNoS prevents impairment of insulin signaling in muscle caused by a high fat diet (Perreault et al., 2001). This induction of SOCS proteins and iNoS represent two additional and potentially important mechanisms that contribute to cytokine-mediated insulin resistance.

Genetic evidence in mice shows that loss of inflammatory mediators or signaling molecules prevents insulin resistance (Hirusumi et al., 2002). Pharmacological targeting of inflammatory pathways using TZDs improve insulin action. Thus the available evidence strongly suggests type 2 DM is an inflammatory disease and the inflammation is a primary cause of obesity-linked insulin resistance, hyperglycemia and hyperlipidemia rather than merely a consequence.

Increased glucose uptake by endothelial cells in hyperglycemic conditions cause excess production of ROS in mitochondria, which inflicts oxidative damage and activates inflammatory signaling cascades inside endothelial cells. Endothelial injury in the adipose tissue might attract inflammatory cells such as macrophages to this site and further exacerbate the local inflammation. Hyperglycemia also stimulates ROS production in adipocytes which leads to increased production of proinflammatory cytokines (Lin et al., 2005).

Schram et al (2003) in reporting the results of this cross sectional analyses of the EURODIAB study, showed that a combined inflammatory Z-score (CRP, TNF-α and IL-6) was associated with retinopathy, albuminuria and CVD.
In recent years, evidence has accumulated that type 2 DM is associated with a subclinical systemic inflammation that might be attributable to a dysregulation of the innate immune system. This immune response is characterized by elevated blood levels of markers of the acute phase response and of its principal mediator IL-6 (Pick up et al., 1998).

There is compelling evidence that augmented levels of IL-6 are associated not only with type 2 DM but also with impaired glucose tolerance (IGT) and predict the development of the disease indicating a potential role of this cytokine in the etiology of type 2 DM (Pradhan et al., 2001; Muller et al., 2002).

Studies of rosiglitazone monotherapy (4-8 mg daily) have demonstrated that the drug is well tolerated, reduces insulin resistance and lower blood glucose in patients with type 2 diabetes (Patel et al., 1999).

Schneider and colleagues observed that for untreated patients whose fasting glucose exceeded 280 mg / dl, daily treatment with pioglitazone (45 mg) for 26 weeks decreased fasting glucose by 71 mg / dl (Schneider et al., 2000).

TZDs resulted in significant reductions not only in fasting glucose levels but also HbA1c and insulin levels (Nolan et al., 2000; Aronoff et al., 2000).

The reduction in levels of proinflammatory markers such as CRP in patients with type 2 diabetes by TZDs indicate the potential beneficial effects of glitazones on overall cardiovascular risk. Fibrinogen, an acute phase reactant is increased in type 2 diabetes and insulin resistance (Mcgill et al., 1994). Rosiglitazone and pioglitazone resulted in a significant decrease in fibrinogen levels (Fonseca et al., 1998).

In the study conducted by Brun et al (1996) decrease in TG, rise in HDL and significant decrease in TC and LDL were observed. TZDs have a direct effect on
adipose tissue which includes enhanced differentiation of preadipocytes into mature adipocytes and regulation of gene expression in adipose tissue leading to a coordinated regulation of lipid metabolism.

Increased levels of markers and mediators of inflammation and acute phase reactants such as fibrinogen, C-reactive protein (CRP), IL-6, plasminogen activator inhibitor (PAI-1), white cell count correlate with incident type 2 DM (Spranger et al., 2003).

Rosiglitazone and pioglitazone are the two currently available TZDs. Both enhance the ability of insulin to transport glucose into skeletal muscle and thus lower circulating insulin levels. (Olefsky et al., 2000). They are also useful for patients with type 2 DM because they decrease hepatic glucose production and prolong pancreatic β cell function by preventing apoptosis of β cell. Both have also been shown to increase HDL-C and reduce triglycerides (Heboritz et al., 2001).

Both the drugs have been shown to reduce blood pressure in animals and humans with Diabetes. Rosiglitazone has also been shown to decrease circulating PAI-1 and CRP levels in patients with diabetes ( Mohanty et al., 2001).

Thus through actions to enhance insulin mediated glucose uptake, through direct effects or both, TZDs improve the metabolic, vasoactive, inflammatory and thrombotic milieu to potentially retard the atherosclerotic process.

Microalbuminuria, a component of metabolic syndrome is an important risk factor for cardiovascular disease in patients with diabetes. (Rius Rin et al., 2003). Among the patients who had metabolic syndrome in this study, pioglitazone significantly decreased the urinary albumin to creatinine ratio by a third, with a significantly greater decrease as compared with findings from metformin monotherapy.

Hyper homocysteinemia is a pathological condition characterized by elevation of homocysteine. Disturbances in the homocysteine metabolism results in a cellular accumulation of homocysteine levels in the circulation. Elevated plasma homocysteine either in fasting state or after methionine load has been proved to be an independent risk factor for vascular disease (Verhaef et al., 1997).

The Framingham offspring study published in diabetes care, 2001 concludes that hyperhomocysteinemia and abnormal urinary albumin excretion are both associated with hyperinsulinemia and account for increased risk of CVD associated with insulin resistance. Because hyperhomocysteinemia and microalbuminuria also reflect endothelial injury, these observations also support the hypothesis that endothelial dysfunction is associated with expression of the insulin receptor substrate (Meigs et al., 2001).

Elevated plasma homocysteine levels have been linked to the development in both arterial and venous vascular disease. The presumed vasculotoxic properties of homocysteine are ascribed to endothelial dysfunction, increased oxidative stress, altered coagulation, smooth muscle cell proliferation and changes in structural as well as elastic properties of the vessel wall (Van Guldener et al., 2000).

A recent study suggests that homocysteine impairs insulin secretion through alterations in β cell glucose metabolism and generation of key stimulus-secretion coupling factors (Patterson et al., 2006).

Elevated Hcy concentration in type 2 DM also suggest an association between homocysteinemia and deterioration of renal function, evidenced by increased serum creatinine and microalbuminuria. These findings implicate homocysteinemia in the
relationship between Diabetic nephropathy and cardiovascular complications of diabetes (Ozmen et al., 2002).

TZDs such as rosiglitazone and pioglitazone can be used more often in patients with type 2 diabetes, because they offer excellent glycemic control as well as decrease in insulin resistance and reductions in cardiac risk factors associated with the insulin resistance. TZDs have positive effects on VSMCs' and assist patients in achieving and maintaining normal HbA₁c levels without the risk of hypoglycemia further reducing cardiac risk. A more prolonged and better glycemic as well as immune control can be expected with the TZDs because of their unique ability to rejuvenate pancreatic β cells.