Review of Literature
Diabetes Mellitus

The term “diabetes” was coined by Aretaeus of Cappadocia (81 – 133 AD) and later on British physician Thomas Willis coined and added “mellitus” (honey sweet) in 1674. Diabetes is a Greek word which means “to pass through or to flow through” and Mellitus means “sweet”. It means a fluid containing sugar passes through the kidneys (glycosuria). The American Diabetes Association (ADA) Expert Committee has defined Diabetes Mellitus as a group of carbohydrate metabolic disorders characterized by hyperglycemia resulting from defects in both insulin secretion and its action. The hormone Insulin is secreted by the β-cells of islet of Langerhans in the pancreas and promotes the utilization of glucose and the storage of excess glucose as glycogen in liver and muscles.

Prevalence of Diabetes

Global

There has been a global increase in the prevalence of diabetes particularly type 2 diabetes\(^{(1)}\). According to International Diabetes Federation (IDF) Diabetes Atlas, 5\(^{th}\) Edition, total number of people with diabetes in world was 366 million in 2011 and is estimated to rise 552 million by the year 2030.

India

Recently International Diabetes Federation (IDF) 2011 has released its report on prevalence of diabetes in different countries. IDF has reported that India have the second largest number of people with diabetes (61.3 million in 2011) in the world and has projected that this number will increase to 101.2 million in by the year 2030. There are major differences in prevalence of diabetes in rural (6.3%) and urban areas (12.1%) (Table 1).
Table 1: Prevalence of Diabetes in Urban and Rural India

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Place</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>Verma et al(^7)</td>
<td>Delhi (Darya Ganj)</td>
<td>3.1*</td>
</tr>
<tr>
<td>1988</td>
<td>Ramachandran et al(^1)</td>
<td>Kudremukh</td>
<td>5.0</td>
</tr>
<tr>
<td>2000</td>
<td>Verma, Madhu(^1)</td>
<td>East Delhi</td>
<td>5.5*</td>
</tr>
<tr>
<td>2001</td>
<td>Misra et al(^5)</td>
<td>New Delhi</td>
<td>10.3</td>
</tr>
<tr>
<td>2001</td>
<td>Mohan et al(^61,62)</td>
<td>Chennai</td>
<td>12.0</td>
</tr>
<tr>
<td>2001</td>
<td>Ramachandran et al(^1)</td>
<td>National Urban Diabetes Survey</td>
<td>12.1</td>
</tr>
<tr>
<td>2004</td>
<td>Sadikot et al(^63)</td>
<td>Urban</td>
<td>5.9</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td>Rural</td>
<td>2.7</td>
</tr>
<tr>
<td>2004</td>
<td>Ramachandran et al(^1)</td>
<td>Rural villages 40 miles away from Chennai</td>
<td>6.3</td>
</tr>
<tr>
<td>2006</td>
<td>Mohan et al(^4)</td>
<td>Chennai</td>
<td>14.3</td>
</tr>
<tr>
<td>2006</td>
<td>Menon et al(^15)</td>
<td>Cochin</td>
<td>19.5</td>
</tr>
</tbody>
</table>

*Known diabetes only

Studies from urban India show a rising trend in the prevalence of diabetes\(^{2,3,5,6,7,}\). Today, the prevalence of diabetes and impaired glucose tolerance (IGT) has reached epidemic proportions\(^{7,10}\) in urban India making it a major national public health problem.

**Classification of Diabetes Mellitus**

Diabetes Mellitus is broadly classified into two major types

1. Type 1 Diabetes Mellitus (T1DM)
2. Type 2 Diabetes Mellitus (T2DM)
However, there are many other rare types of diabetes exist as well.

**Type 1 Diabetes Mellitus**

Type 1 diabetes, earlier called insulin dependent diabetes mellitus (IDDM), results from lack of insulin secretion by the pancreas, caused by complete destruction of $\beta$-cells.

**Type 2 Diabetes Mellitus**

Type 2 diabetes, earlier called non-insulin dependent diabetes mellitus (NIDDM), is more common type and accounts for almost 90-95% of diabetes in our country.

**Diagnosis of Diabetes**

There are three ways of diagnosing diabetes and each must be confirmed on subsequent day unless classic symptoms of hyperglycemia are present.

1. Classical symptoms of diabetes and casual plasma glucose concentrations $\geq 200$mg/dl.
   
   Casual means any time of the day regardless of time since last meal.
   
   OR

2. Fasting plasma glucose (FPG) $\geq 126$ mg/dl

   OR

3. 2 hour plasma glucose (2h PG) $\geq 200$ mg/dl during an oral glucose tolerance test (OGTT).

**Symptoms**

The classical symptoms of diabetes mellitus are

- Excessive thirst (polydipsia)
- Frequent urination (polyurea)
- Increased appetite (polyphagia)
- Rapid weight loss

**Risk Factors of Diabetes**

Among the important risk factors reported for the high prevalence of diabetes in India are high familial aggregation, racial predisposition, obesity particularly central obesity, insulin resistance and life style changes associated with urbanization$^{(9,15,16,17,37)}$.  

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*Department of Medicine*
Familial Aggregation: The prevalence of diabetes among offspring with one diabetic parent was 36%. When both parents are diabetic the prevalence rate increased further (62%)\(^{(38)}\). There have been several studies that have focused on altered dietary and physical activity patterns associated with urbanization\(^{(9,39,40,41)}\), that have also contributed to a rise in diabetes prevalence.

Racial Predisposition: Asian population living in different parts of the world has higher prevalence of diabetes as compared with the co-inhabitants of other races\(^{(42)}\). Recent studies have demonstrated that even internal migration within a country, causing more affluence and sedentary lifestyle un masks the tendency for diabetes in the Asian races\(^{(12)}\).

Physical Activity: Physical activity is probably a protective factor against development of diabetes\(^{(43)}\). Studies of Mohan and colleagues have documented that the prevalence of diabetes was higher among subjects with light grade (17%) compared to moderate grade (19.7%) and heavy grade physical activity (5.6\%)\(^{(44)}\).

Stress: Stress (oxidative stress as well as environmental stress) has also emerged as an important risk factor in the development of type 2 diabetes mellitus. Studies from our own institute and several other studies have shown the possible role of stress in causation of type 2 diabetes mellitus\(^{(45,219)}\).

Urbanization: Growing industrialization is motivating peoples to migrate from rural to urban areas. Ramachandran and colleagues have reported that prevalence of diabetes in semi-urban area was found to be 5.9% in comparison with prevalence of 2.4% in rural and 11.6% in the urban population\(^{(15)}\). The changes in socioeconomic status in rural India have produced significant changes in lifestyle. Sedentary lifestyle and increasing rate of obesity are significantly associated with the increasing prevalence of glucose intolerance.

Diet: Diet and nutrition are believed to play an important role in the development of Type 2 diabetes mellitus. Findings indicate that a higher intake of polyunsaturated fat and possibly long-chain n-3 fatty acids could be beneficial\(^{(46)}\), whereas a higher intake of saturated fat and trans-fat could adversely affect glucose metabolism and leads to the development insulin resistance. In contrast, a low-glycaemic index diet, higher amount of
fiber and whole grain products reduce the risk of type 2 diabetes\textsuperscript{(47)}. Also, long term intake of high sucrose or high fructose diets or diets rich in fat content are known to cause insulin resistance\textsuperscript{(48,49,50)}. Therefore type of diet and nutrition are believed to be an independent and important risk factor in the development of type 2 diabetes.

**Glucose Homeostasis**

1. **Metabolic Response to Glucose under Normal Conditions**

   The metabolic response to ingested carbohydrate is markedly different in individuals with NGT compared to those with T2DM. Individuals with normal glucose metabolism have a typical glucose, insulin and glucagon profile in plasma in response to the ingestion of a carbohydrate meal\textsuperscript{(51)}. After ingestion, there is an expected increase in plasma glucose levels as well as a robust insulin response to dispose off the ingested glucose load. However, in contrast, glucagon secretion is suppressed and plasma levels of glucagon decrease\textsuperscript{(51)}, leading to the inhibition of endogenous glucose production. This interplay represents the ideal response of pancreatic $\alpha$ and $\beta$ cell types. This interplay of insulin and glucagon allows plasma glucose to remain within a very narrow homeostatic range despite the wide variation in food intake that occurs with the transition from the fasting to the post prandial state. Both, an increase in insulin secretion and the decrease in glucagon levels are required to promote optimal storage and disposal of glucose\textsuperscript{(51)}. Thus, insulin and glucagon represent a key set of counterregulatory hormones involved in the metabolic response to glucose.
2. Metabolic Response to Glucose in Type 2 Diabetes

With the development of impaired glucose tolerance and its progression to T2DM, the metabolic response to ingested glucose changes dramatically\(^{(52)}\) leading to the onset of hyperglycemia, a prime manifestation of T2DM. This hyperglycemia is a consequence of insulin resistance in peripheral tissues as well as inadequate secretion of insulin and an impaired suppression of glucagon secretion in response to ingested glucose. After a meal (post prandial state), plasma glucose levels increase in normal subjects as expected and
those with T2DM. However, in T2DM patients this increase is more pronounced. In addition to this, the spike in plasma insulin is noticeably blunted and delayed in patients with T2DM. Also, in contrast to normal subjects, the decrease in glucagon levels does not occur and in fact, a slight increase is observed in glucagon levels. This absence of decrease in glucagon levels is one of the key factors in understanding the glucose dysregulation, insulin resistance, and insufficient insulin production that occur in patients with T2DM\(^{(52)}\).

**Figure 2: Plasma Glucose, Insulin and Glucagon Profiles in Response to Ingestion of a Carbohydrate Meal in Normal Subjects and Patients with T2DM**

Pathogenesis of Type 2 Diabetes Mellitus

It has long been understood that the pathophysiology of type 2 diabetes is based on three important components (a) Increase in insulin resistance (b) Progressive decline in insulin-producing pancreatic β cells and (c) Increased hepatic glucose production\(^{(53,54)}\).

Type 2 diabetes involves at least two primary pathogenic mechanisms:

1. Insulin resistance resulting in a decrease in the metabolic responses to insulin\(^{(55,56)}\).
2. Progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and inadequate suppression of glucagon secretion\(^{(52,55)}\).
Third pathogenic mechanism involving gastrointestinal hormones - incretins, has also been postulated recently. However, this mechanism has not been fully understood so far.

1. Pathogenic Mechanism of Insulin Resistance

The concept of insulin resistance was given by Himsworth in the 1930s, which he observed in diabetes patients who did not respond to insulin treatment\(^{(57)}\). Insulin resistance is a common factor of many metabolic diseases\(^{(58,59)}\) as well as a central component of metabolic syndrome which is also known as “syndrome X” or “the insulin resistance syndrome”. In 1998 World Health Organization (WHO) provided definition of the metabolic syndrome that includes insulin resistance, impaired glucose tolerance, as a necessary component combined with at least two among obesity, dyslipidemia, hypertension and microalbuminuria\(^{(60)}\). A number of studies in humans as well as in animals have been carried on insulin resistance and our knowledge has increased considerably in this context. However, the underlying mechanisms of insulin resistance have not been fully understood.

Insulin resistance is an ultimate predictor of the type 2 diabetes development and together with decreased insulin secretion from the β-cells of the pancreas it provides the perfect pathophysiological basis for the development of type 2 diabetes\(^{(58)}\). Initially, β cells compensate for insulin resistance by increasing insulin secretion that results in the development of hyperinsulinemia. However, later on as time goes by, the β cell function diminishes and these cells fail to compensate for increasing insulin resistance in target tissues viz muscles, adipose tissue and liver. Therefore, hyperglycemia manifests\(^{(61)}\) and and eventually type 2 diabetes develops.
Insulin and Glucose Turnover

The β-cells of the pancreas respond to increasing glucose levels by releasing insulin into the blood. Insulin affects glucose turnover in many tissues. In liver, insulin inhibits glycogenolysis and gluconeogenesis\(^{(62)}\). In skeletal muscle, which accounts for approximately 75% of insulin-mediated glucose clearance after a glucose challenge\(^{(63,64)}\), insulin promotes glucose uptake into cells. Insulin is involved in the regulation of glycogen synthase as well as key enzymes in glycolysis. Apart from muscles, insulin has a potent effect to inhibit adipose tissue lipolysis and the release of glycerol and free fatty acids (FFAs) into the blood and this step is of great importance for glucose homeostasis.

Increased levels and oxidation of FFAs are thought to contribute to the development of muscle insulin resistance\(^{(65,66)}\). In liver, FFAs blunt insulin’s effects on hepatic glucose metabolism, and increase endogenous glucose production both by stimulating key enzymes and by providing energy for gluconeogenesis\(^{(67)}\). Moreover, glycerol released during triglyceride hydrolysis serves as a gluconeogenic substrate\(^{(68)}\). Consequently,
resistance to the antilipolytic action of insulin in adipose tissue results in excessive release of FFAs and glycerol that may have deleterious effects on glucose turnover and homeostasis.

**Insulin and Lipid Turnover**

One of insulin’s most potent metabolic actions in respect of lipid homeostasis is suppression of adipose tissue lipolysis\(^{[69,70,71]}\). This antilipolytic effect of insulin is mediated through inhibition of hormonesensitive lipase (HSL). Upon insulin stimulation, phosphodiesterase 3B (PDE3B) is activated leading to a reduction of the intracellular cAMP level and this in turn attenuates the activity of cAMP-dependent protein kinase A (PKA) responsible for phosphorylation and activation of HSL. This cascade of events leads to the clearance of triglyceride-rich lipoproteins (TRLs) from blood\(^{[72,73]}\). In contrast, skeletal muscle LPL activity is decreased in the postprandial state, possibly by the increase in glucose and/or insulin levels\(^{[74]}\). The opposite effects of insulin on regulation of LPL activity in adipose tissues and muscles, probably serves to divert FFAs derived from triglyceride-rich lipoproteins away from muscle and to adipose tissue for lipid storage. In mature adipocytes, insulin promotes triglyceride storage by stimulating glucose uptake and conversion of acetyl-CoA into triglycerides as well as by inhibiting lipolysis. Insulin also increases the cellular uptake of fatty acids\(^{[70]}\) derived from circulating lipoproteins by stimulating lipoprotein lipase activity in the vasculature of adipose tissue.

**Lipocentric Pathway**

The exponential rise in the prevalence of type 2 diabetes mellitus (T2DM) and metabolic syndrome in recent years in developed as well as developing nations is believed to be a consequence of increasing sedentary life styles and ready access to high calorie food products. Healthy subjects respond to this condition by showing positive energy balance by storing excess calorie in the form of triglyceride in adipose tissue resulting in weight gain and obesity. Persistent over nutrition in these individuals leads to saturation of these adipose depots after which accumulation of fat in “ectopic sites” in visceral depots such
as liver, skeletal muscles, pancreas and kidney occurs. This causes lipotoxicity at these sites and through a cascade of events results in increased insulin resistance, β cell dysfunction and diabetes. This overflow of energy together with alterations in carbohydrate and lipid metabolism is referred to as “lipocentric hypothesis” of the pathogenesis of insulin resistance and T2DM.

Evidence for the lipocentric hypothesis comes from the clinical study which was carried out by Kim and colleagues to analyze how fat partitioning in the liver, muscle and visceral compartments is altered by diabetes and age, and whether altered fat distribution is associated with a higher carotid artery intima-media thickness (C-IMT) and insulin resistance. Results of this study showed that older as well as young diabetes patients had higher visceral fat areas, higher liver attenuation and higher lipid-rich muscle when compared with healthy individuals. They also concluded that high fat stores within ectopic compartments were observed at an early stage in the development of diabetes. Furthermore, altered lipid partitioning within muscle was independently associated with carotid atherosclerosis and insulin resistance\(^{(75)}\).

One more study which support lipocentric hypotheses and suggests that visceral fat accumulation leads to insulin resistance is of Ilan and co-workers, aimed to investigate whether the accumulation of visceral fat (VF) could play a direct role in the pathophysiology of insulin resistance and type 2 diabetes. They monitored insulin action, glucose tolerance and the expression of adipo-derived peptides after surgical removal of VF in aging (20-month-old) F344/Brown Norway (FBN) and in Zucker Diabetic Fatty (ZDF) rats and demonstrated that removal of visceral fat restore peripheral and hepatic insulin action to the levels of young rats. Furthermore, they observed that removal of visceral in ZDF rats prevented the progressive decrease in insulin action and delayed the onset of diabetes. This study documents a cause-and-effect relationship between VF and insulin resistance\(^{(76)}\).


Figure 5: Cell Biology of Insulin Response in Normal and Diabetes State

<table>
<thead>
<tr>
<th></th>
<th>Normal Cell</th>
<th>Type I Diabetes Cell</th>
<th>Type II Diabetes Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
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<td><img src="image2" alt="Image" /></td>
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<tr>
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<td><img src="image5" alt="Image" /></td>
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<tr>
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<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td>Closed Glucose Transporter</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
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<tr>
<td>Open Glucose Transporter</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
</tr>
</tbody>
</table>

A. This is a normal cell. Insulin is present and is taken into the cell to facilitate proper glucose uptake and metabolism.

B. In Type I diabetes, insulin is not produced; so, there is nothing to signal the cells to take in glucose and metabolize it.

C. In Type II diabetes, insulin is present, but the signal for proper glucose uptake and metabolism is lost. The problem could be in the insulin itself or in any one of the proteins involved in glucose uptake and metabolism.

2. Pathogenic Mechanism of Islet Cells

Located in the pancreas, islets of Langerhans (endocrine cells of the pancreas) are primarily composed of two types of cells: α cells and β cells. However, variations in islet cell architecture in terms of cell quantity and location is seen between the species for example in rodents, β cells are clustered in the core of the islet and α cells are localized to the periphery of the islet and in case of humans, α and β cells are found scattered throughout the islet along blood vessels in association with microcirculation.
The β cells comprise approximately 60% of the endocrine mass of the pancreas\(^{(77)}\) and produce both insulin and amylin, insulin is released in response to elevation in plasma glucose levels. The α cells comprise about 30% of the endocrine mass of the pancreas, secrete glucagon in response to decreases in plasma glucose levels\(^{(77)}\).

**Pancreatic Endocrine Cells, Insulin Secretion and Glucagon**

The pathogenic mechanisms in T2DM involve not only insulin, but also glucagon, and it is the interplay between these two processes that is a key component in the understanding of the pathophysiology of T2DM.

One key reason underlying the profound postprandial hyperglycemia observed in patients with T2DM is a decrease in the peripheral uptake of glucose. In response to increasing insulin levels in normal individuals as well as those with T2DM, peripheral uptake of glucose occurs. However, the efficiency of uptake in patients with T2DM is markedly reduced. According to Groop and co-workers the rate of total body glucose disposal as a function of increasing insulin concentrations showed the rate of glucose uptake was reduced by about 30% in patients with T2DM\(^{(78)}\). They also showed that fasting plasma glucose (FPG) was significantly correlated with basal HGP. These findings suggest that the level of insulin production achieved in a patient with T2DM is insufficient to control hyperglycemia. Patients with T2DM also have a reduced uptake of glucose into cells, less efficient shunting of glucose into key metabolic pathways such as the tricarboxylic acid cycle, and less storage of glycogen\(^{(79,80)}\).

Another key reason for hyperglycemia particularly postprandial glucose elevation in T2DM relates to the blunted glucose-stimulated production of insulin. While many potential mechanisms may account for this phenomenon, the reduction of pancreatic β cell mass in patients with T2DM is worth noting. Also, results from autopsy studies of obese individuals with NGT, IFG or T2DM showed that, with progression from NGT to IFG to T2DM, β cell masses progressively decrease\(^{(81)}\). Patients with IFG had a 40% decrease in relative β cell volume, whereas those with T2DM had a 63% decrease,
compared to the NGT group\(^{(81)}\). Thus, one reason for low insulin and, consequently, hyperglycemia, in patients with T2DM is the progressive loss of pancreatic β cells. Similar results have been reported in other clinical trials, in which not only has pancreatic islet cell mass decreased in patients with T2DM, but the remaining islets do not function properly. Islets from the pancreas of cadaveric T2DM and normal cadaveric donors were isolated and matched for age, body mass index, and cold ischemia time. A significant decrease in islet cell mass between patients with T2DM and normal donors was observed\(^{(82)}\). Moreover, patients with T2DM had poorer islet function compared to controls and the threshold for glucose-stimulated insulin release (GSIR) was much higher in type 2 diabetes patients (7 and 12 mmol/L, normal vs diabetic islets)\(^{(82)}\). These findings suggest a loss of islet mass and an apparent decline in islet cell function in patients with T2DM.

3. Hepatic Glucose Production and the Incretin Effect

The key metabolic defects associated with T2DM are decreased insulin production and secretion, increased insulin resistance, and continued production of glucagon. One other key component of hyperglycemia associated with T2DM is the basal rate of glucose production by the liver. Basal hepatic glucose production (HGP) in patients with T2DM is markedly increased\(^{(78)}\) compared to subjects with normal glucose metabolism. Therefore, in addition to less efficient postprandial glucose transport and metabolism, patients with T2DM also have elevated production of glucose by their liver, resulting in markedly elevated postprandial plasma glucose levels\(^{(78)}\).

The possible mechanism of increased HGP is the process by which hepatic gluconeogenesis is switched off, particularly; a deficiency in glucagon production which is responsible for profound and sustained reduction in HGP. A clinical study conducted by Liljenquist group, reported that when healthy non-obese men were treated with an infusion of somatostatin to suppress endogenous plasma insulin and glucagon, glucagon levels were reduced by more than 50% in these subjects so as HGP reduced by 75%. Furthermore this remained suppressed throughout the study\(^{(83)}\). This study highlight the
importance of reducing glucagon levels to suppress HGP in patients with T2DM as well as the important role of glucagon in sustaining HGP in normal subjects. In T2DM, postprandial glucagon secretion is not suppressed as it is in case of normal subjects. Therefore, the net result of elevated glucagon levels is that the liver will continue to produce glucose, regardless of its requirement(52).

**Incretins**

Existence of incretins was postulated in early 1900s when investigators first observed a fundamental difference in the plasma insulin response when equimolar amounts of glucose were administered by the oral and intravenous (i.v.) route. The term *incretin effect* refers to the difference between these two plasma insulin profiles. This differential effect of oral vs i.v. glucose administration suggested the existence of substances in the gut that mediate this response and led to the discovery of *incretin hormones*. Moreover, key role for these incretins in the pathogenesis of diabetes is suggested by the finding that the incretin effect is substantially blunted in patients with T2DM(84). Two major incretins secreted in the gut are;

1. Glucagon-like peptide-1 (GLP-1)
2. Glucose-dependent insulinotropic peptide (GIP)

Significant role of these gastrointestinal hormones (GLP-1 and GIP) is being increasingly recognized(54,85,86). The dual action of GLP-1 in both stimulating insulin secretion as well as inhibiting glucagon secretion highlights its potential benefit in the treatment of two key metabolic abnormalities associated with T2DM. Also, the exogenous administration of GLP-1 to patients with poorly controlled T2DM has been shown to normalize plasma glucose levels and reduce glucagon secretion(87).

**Gastrointestinal System and Glucose Homeostasis in Healthy State**

The GI system plays an integral role in glucose homeostasis(88). The observation that orally administered glucose provides a stronger insulinitropic stimulus than an
Intravenous glucose challenge (*incretin effect*) provided insight into the regulation of plasma glucose by the GI system of healthy individuals\(^{89}\). The *incretin effect* may be responsible for 50% - 70% of the total insulin secretion following oral glucose intake\(^{90}\).

Incretins are known to exert major glucoregulatory actions\(^{91,92,93}\) within minutes of nutrient ingestion. Additionally, GLP-1 has been shown to inhibit glucose-dependent glucagon secretion from \(\alpha\) cells\(^{91}\).

Under healthy conditions, fasting glucose is managed by insulin/glucagon secretion, but postprandial glucose (PPG) is controlled by insulin and the incretin hormones\(^{84}\). Additionally, in animal studies, GLP-1 has been shown to induce the transcriptional activation of the insulin gene and insulin biosynthesis, thus increasing \(\beta\) cell proliferation and decreasing \(\beta\) cell apoptosis\(^{94}\). Baggio has reported some other important functions of GLP-1 viz stimulation of CNS-mediated pathway of insulin secretion, slow gastric emptying, increased CNS-mediated satiety leading to reduced food intake, indirectly increased insulin sensitivity and nutrient uptake in skeletal muscle and adipose tissue, and neuroprotective effects\(^{90}\). Apart from its insulinotropic action, GIP has been shown in animal studies to inhibit gastric acid secretion, bioregulate fat metabolism in adipocytes, increased glucagon secretion and fat deposition, increased \(\beta\) cell replication, and decrease in \(\beta\) cell apoptosis\(^{90}\).

### Incretins and T2DM

Studies have shown that incretin pathways play a role in the progression of T2DM\(^{84,91}\). The significant reduction of incretin effect evident in patients with T2DM has been attributed to several factors, including impaired secretion of GLP-1, accelerated metabolism of both GLP-1 and GIP, and defective responsiveness to both hormones\(^{84}\). Many patients with T2DM also have accelerated gastric emptying that may contribute to deterioration of their glycemic control\(^{95}\).
One more significant finding with respect to GLP-1’s role in the development of diabetes comes from experimental study in obese diabetic mice which suggest that the effect of GLP-1 therapy on the long-term remission of diabetes may be caused by improvements in \( \beta \) cell function and insulin sensitivity, as well as by a reduction in gluconeogenesis in the liver\(^{(96)}\).

Another study conducted to evaluate quantitatively the separate impacts of obesity and hyperglycemia on the incretin effect in patients with T2DM, subjects with impaired glucose tolerance and subjects with normal glucose tolerance\(^{(97)}\), reported significant reduction in the incretin effect in terms of total insulin secretion, \( \beta \) cell glucose sensitivity and the GLP-1 response to oral glucose in patients with T2DM compared with individuals whose glucose tolerance was normal or impaired.

Exogenous GLP-1 has been shown to restore the regulation of blood glucose to near-normal concentrations in patients with T2DM\(^{(98)}\). Several studies of patients with T2DM have shown that synthetic GLP-1 administration induces insulin secretion\(^{(87,98)}\) slows gastric emptying (which is accelerated in patients with T2DM) and decreases inappropriately elevated glucagon secretion\(^{(87,99)}\). Acute GLP-1 infusion studies showed that GLP-1 improved fasting plasma glucose (FPG) and post prandial glucose (PPG) concentrations\(^{(87,98)}\). Long-term studies showed that this hormone exerts euglycemic effects, leading to improvements in glycosylated hemoglobin (HbA1c) and induces weight loss\(^{(100)}\).
Figure 6: Biologic Actions of GIP and GLP-1 in Relation to the Pathophysiology of Type 2 Diabetes Mellitus

*solid arrows = potentially beneficial actions; dashed arrows = potentially harmful actions; slashed arrows = actions with no effect

Evidences Suggesting Role of Post Prandial Lipaemia/Post Prandial Hypertriglyceridemia in the Development of T2DM and Insulin Resistance

Dyslipidemia is an established defect in type 2 diabetes patients. There is a great deal of data that suggest that T2DM patients show increased lipid levels. However, in last two decade, post prandial lipid abnormalities have been in focus with respect to T2DM. Some of recent findings indicate that PPHTg may be an early event leading/contributing to the development of T2DM.

Results of the prospective population study with 10 years of follow up in subjects with clustering of endogenous hypertriglyceridemia have shown that these subjects are at increased risk of type 2 diabetes. They have also reported that in these subjects elevation of serum TG serves as a risk marker of glucose intolerance and type 2 diabetes. Further, T2DM and IR have been shown to be associated with hypertriglyceridemia\(^{101,102}\).

A cross sectional study conducted by Axelsen and co-workers in normotriglyceridemic men with two first-degree relatives with type 2 diabetes and matched controls without known diabetes heredity have shown that relatives of persons with type 2 diabetes were insulin resistant but had normal glucose tolerance. This postprandial hypertriglyceridemia was prevalent despite the fact they had normal fasting triglyceride levels\(^{103}\). Since elevated FTG may actually be a consequence of post prandial hypertriglyceridemia\(^{104}\). It is likely that high post prandial TGs carry higher risk of future T2DM.

When post prandial lipid responses following oral fat challenge were studied in subjects with NGT, pure IFG, pure IGT and newly detected diabetes mellitus patients, there was significantly higher post prandial triglyceride response with a higher post prandial triglyceride area under the curve and peak post prandial TG levels in newly detected diabetes mellitus patients. Also, obese individuals demonstrate higher post prandial TG levels than non-obese individuals\(^{33}\).
Similar study was also conducted in first degree relatives of type 2 diabetes patients with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) subjects. Results of this study showed the presence of post prandial lipaemia indicating that it is an independent inherited defect which occurs prior to the development of T2DM\(^{(105)}\).

The most direct human evidence of the causal role of TGs in T2DM comes from the case reported by Mingrone where, two sisters had extreme hypertriglyceridemia and overt diabetes. In these siblings, medical therapy (including high-dose insulin) failed to reduce plasma TGs or control glycemia, lipid malabsorption was surgically induced by a modified biliopancreatic diversion. Within 3 weeks of surgery, plasma TGs and NEFA and cholesterol levels were drastically lowered. Concurrently, fasting plasma glucose levels fell from 17 to 5 mmol/l (with no therapy). Thus, surgical normalization of TGs cured the diabetes and near complete reversal of insulin resistance occurred. Also, insulin-stimulated glucose uptake, oxidation, and storage were all markedly improved. Throughout the observation period, plasma TG levels were closely correlated with both plasma glucose and insulin concentrations, as measured during the oral glucose tolerance test. These cases study provide the evidence that insulin-resistant diabetes can be caused by extremely high levels of TGs\(^{(106)}\).

The postprandial levels of triglyceride-rich lipoproteins and their remnants are shown to be elevated in type 2 diabetes\(^{(107,108)}\). Epidemiological data also suggest that abnormal clearance of triglyceride-rich lipoproteins in the postprandial phase is an early defect in type 2 diabetes as healthy first degree relatives of patients with type 2 diabetes exhibit postprandial hypertriglyceridaemia despite having normal fasting triglyceride levels\(^{(103)}\).

Much more controversy arises from the study conducted by Rivellese Angela, where they evaluated exogenous and endogenous lipoprotein responses to a fat rich meal in type 2 diabetic patients who had optimal fasting triglyceride levels and optimal blood glucose control. Researchers found that triglyceride incremental areas were significantly higher in
diabetes patients and so as the increase in large very low density lipoproteins of both endogenous and exogenous origins\(^{109}\).

Results of the experimental study with 22 week long follow up, carried out by Yutaka Mori and co-workers in Otsuka Long-Evans Tokushima Fatty (OLETF) rats showed, improved lipid metabolism, glucose tolerance and retarded progression of diabetes when post prandial lipaemia was blunted by dietary source (diacylglycerol) in these rats\(^{110}\).

**Post Prandial Lipaemia and Insulin Resistance**

There is considerably good number of studies that have shown relationship of PPHTg and insulin resistance. However, whether insulin resistance is a consequence of PPHTg or a cause remains controversial. Results of some of the studies suggest that PPHTg may be a contributing factor in the progression and development of insulin resistance.

Bergstrom and co-workers reported from a clinical study of offsprings of T2DM patients that intramyocellular lipid (TG) accumulation following excess triglycerides in blood leads to muscles insulin resistance and this abnormality precedes the development of hepatic insulin resistance and T2DM\(^{111}\).

Pedrini and his colleagues studied the effect of purified TGRLs on glycogen synthesis, glycogen synthase activity, glucose uptake, insulin signalling and intramyocellular lipid (IMCL) content using fully differentiated L6 skeletal muscle cells (an in vitro study). They reported that incubation with TGRLs diminishes insulin-stimulated glycogen synthesis, glycogen synthase activity, glucose uptake and insulin-stimulated phosphorylation of Akt and glycogen synthase kinase 3 independent of NEFA levels and suggested that the accumulation of TGRLs in the blood stream of insulin-resistant patients may not only be a consequence of insulin resistance but could be a cause\(^{112}\).
PPAR-γ agonists which are known to decrease PPHTg, have been shown to decrease the incidence of T2DM significantly when given to IGT subjects and also improve hepatosteatosis as well as hepatic insulin sensitivity. In addition, experimental studies in rats have also confirmed that intervention with Thiazolidinediones reduces insulin resistance\textsuperscript{(113,114)}.

Savage and co-workers, three patients with dominant-negative mutations in the nuclear hormone receptor peroxisome proliferator-activated receptor (PPAR)-gamma. They reported that humans with dominant mutational function in adipose tissue in the ligand binding domain of PPAR-γ manifest a stereotype form of partial lipodystrophy and impaired post prandial fatty acid trapping with resulting increase in PPTGs, fatty liver disease and severe insulin resistance\textsuperscript{(114)}.

Experimental study carried out in sprague-Dawley rats to assess adipose lipoprotein lipase (LPL) resistance to insulin in a nutritional model of resistance of glucose metabolism to insulin. Results of this study showed that Expression of enzymes related to lipid turnover in visceral fat (e.g. LPL, hormone sensitive lipase and PPAR-γ ) increase compared to subcutaneous fat (SF) with fat feeding, which leads to increased FFA flux to liver causing increased hepatic fat content (TG accumulation) and hepatic IR\textsuperscript{(115)}.

An observational study conducted in young healthy men and men with newly developed type 2 diabetes and 35 middle-aged subjects and pattern of fat distribution was studied. Authors reported that the young and middle-aged diabetic subjects had higher visceral fat areas, higher liver attenuation and higher lipid-rich muscle when compared with healthy individuals. Moreover, high fat stores within ectopic compartments were observed at an early stage of development of diabetes as well as in insulin resistant non-diabetic obese adolescents\textsuperscript{(75)}.

Results of a cross sectional study in healthy subjects showed a decrease in insulin sensitivity during postprandial lipaemia and suggested that decreased insulin sensitivity is
a consequence of elevated plasma levels of triglyceride-rich lipoproteins independently of plasma NEFA levels\(^{116}\).

Experimental studies carried out to study fat distribution in vivo as well as in vitro in mice and rat feeding high fat diet have shown suppression of PPHTg by dietary supplements and so as decrease in visceral fat deposition\(^{(47,117,118)}\).

Experimental study carried out by Toyodo and co-workers in rats and mice to study the suppressive effects of oolong tea extract on postprandial hypertriglyceridemia have shown that blunting of post prandial hypertriglyceridemia is an useful in the prevention of obesity\(^{(119)}\).

Clinical study carried out in normotriglyceridemic subjects with impaired glucose tolerance and with normal glucose tolerance by Higashi and co-workers shows that post prandial TG responses may be associated with early-phase insulin secretion and without insulin resistance in normotriglyceridemic men with IGT or NGT. However, in this study they did not observe any increase in post prandial TG response in IGT subjects\(^{(120)}\).

Karamanos and co-workers studied post prandial hypertriglyceridaemia and its relation with insulin in healthy subjects and measured glucose, insulin and triglycerides in fasting and 1, 2, 3 and 4 h after a high fat low carbohydrate meal. Results of this study showed that (a) post prandial hypertriglyceridaemia can occur irrespectively of the fasting triglyceride concentrations. (b) A percent increase over fasting value (PTI) of ≥ 80% is associated with a significant increase of insulin resistance\(^{(121)}\).

Experimental studies carried out in high sucrose fed rats challenged with olive oil showed that post prandial hypertriglyceridemia is accompanied by hyperinsulinemia\(^{(122)}\), impaired glucose tolerance\(^{(123)}\), increased VLDL-TG secretion\(^{(124)}\), and a decrease in both lipoprotein lipase and hepatic TG lipase activity\(^{(56,125)}\). Further investigations in these rat models also indicate that a sucrose rich diet produces not only an increase in the rate of triglyceride secretion in TRLs but also a decrease in the clearance rate of triglyceride rich emulsions in post prandial lipaemic conditions reflecting the clinical manifestations of post prandial lipaemia.
Short term experimental study carried out by Samuel and colleagues in Sprague-Dawley rats shows that high fat feeding elevates liver triglycerides in these rats causing hepatic insulin resistance\(^{(126)}\).

**Atherosclerosis**

The term atherosclerosis was coined by German Pathologist Felix Jacob Marchand in 1904. The word atherosclerosis comes from Greek word “athero” meaning paste and “sclerosis” meaning hardness. This term is used for the process of fatty substances, cholesterol, cellular waste products, calcium and fibrin accumulating in the inner lining of an artery. The buildup body that results is called plaque. This plaque may block the flow of blood through an artery partially or completely.

Two things that can happen where plaque occurs are:

1. There may be bleeding (hemorrhage) into the plaque.
2. A blood clot (thrombus) may form on the plaque's surface.

If either of these two conditions occurs and blocks the whole artery, a heart attack or stroke may result. Atherosclerosis affects large and medium-size arteries. The type of artery and the site where the plaque develops varies from person to person.

Atherosclerosis is a slow, progressive disease that may start in childhood. In some people this disease progresses rapidly in their third decade of age whereas in others it doesn't become threatening until they're in their 50s or 60s years of life.

**Prevalence**

Cardiovascular disease (CVD) has emerged as the leading cause of death all over the world as well as in India. Coronary heart disease (CHD) affecting Indians at least 5-6 years earlier than their western counterparts\(^{(127,128)}\). The estimates from different cross-
sectional studies show the prevalence of CHD to be between 7-13 per cent in urban and 2-7 per cent in rural India\(^{(129)}\).

**Pathogenesis of Atherosclerosis**

This process affects medium and large-size arteries and is characterized by patchy thickening of the subintima that encroaches on the arterial lumen and any of the vascular bed may be affected by this process. The etiology treatment and clinical impact of atherosclerosis varies from one vascular bed to other\(^{(24)}\).

The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden foam cells in the intimal layer of the artery. With time, the fatty streak evolves into a fibrous plaque, the characteristic of established atherosclerosis. Ultimately the lesion may evolve to contain large amounts of lipid.

Atherosclerotic lesions are composed of three major components;

1. Smooth muscle cells and macrophages
2. Connective tissue matrix and extracellular lipid
3. Intracellular lipid that accumulates within macrophages converting them into foam cells

**Role of Inflammation, Endothelial Perturbation and Lipid**

Various cytokines, smooth muscle cells proliferation, synthesis of connective tissue matrix, and accumulation of macrophages and lipids contribute to the development of atherosclerotic lesions. The earliest stages of atherosclerosis are characterized by perturbation in endothelial function and most likely initiate when endothelial cells overexpress adhesion molecules in response to dyslipidemia. This finding was confirmed by Li and co-workers when they demonstrated that rabbits fed on a pro-atherogenic diet rapidly overexpress vascular cell adhesion molecule-1 (VCAM-1) and said that
expression of VCAM-1 on endothelial surfaces is an early and necessary step in the pathogenesis of atherosclerosis\(^\text{(130)}\). Increased cellular adhesion and associated endothelial dysfunction then “sets the stage” for the recruitment of inflammatory cells, release of cytokines and accumulation of lipids into the atherosclerotic plaque.

In view of these facts, inflammatory markers particularly high sensitive C-reactive protein (HsCRP) and fibrinogen are being considered of great importance. Experimental studies also support association of these markers with pathogenesis of atherosclerosis. Danenberg and his coworkers have carried out experimental studies and evaluated the proinflammatory and prothrombotic effects of CRP on monocytes and endothelial cells in vivo in wild type mice which do not express CRP, and human CRP-transgenic (CRPtg) mice models of arterial injury. In an arterial injury model complete thrombotic occlusion of the femoral artery at 28 days was seen in 17% of wild-type mice compared with 75% of CRPtg arteries\(^\text{(131)}\). Even after adjustment for lipid status, CRP levels remain independent predictors of atherosclerosis, including peripheral arterial disease\(^\text{(132)}\).

**Role of Lipid**

The lipocentric hypothesis of atherosclerosis has changed dramatically over the last 2 decades. Once viewed as the initiating agent of atherothrombosis, it is now being recognized that localization and accumulation of lipid occurs in response to earlier changes in the vascular endothelium. However, accumulation of lipid is anyway required for the development of the definitive plaque. Deposition of lipid starts possibly with the movement of LDL from the blood into the vessel wall. Once within the media three possibilities of LDL fate are there;

1. LDL move back into the bloodstream (lesion regression process that may be facilitated by some lipid lowering strategies)
2. Oxidation of LDL (through action of free radicals or direct activity of leukocytes)
3. LDL is taken up by monocyte/macrophages which ultimately become foam cells.

Uptake of oxidized LDL leaves the macrophages less mobile, therefore promoting the accumulation of these lipid laden cells in the intima. At this stage foam cells retain their metabolic activity and secrete a variety of cytokines and inflammatory mediators.

**Development of the Atherosclerotic Plaque**

Progression of the atherosclerotic plaque is characterized by gradual enlargement over time due to the accumulation of foam cells. Some plaques grow at much greater speed than would be predicted by simple lipid accumulation and expansion of the components of the fibrous plaque. Cholesterol accumulation within such plaques is due to both “passive” transfer of LDL from the circulation and scavenging of red blood cell membranes deposited during intraplaque hemorrhage\(^{(133)}\).
Evidences that Post Prandial Lipaemia/Hypertriglyceridemia Contributes in the Development of Atherosclerosis

Several lines of evidence support the concept that post prandial lipaemia particularly post prandial hypertriglyceridemia could be an important factor in the development of diabetes related atherosclerosis. Studies that have shown association of PPL with diabetes related atherosclerosis include:

Data from the clinical studies shows that the relationship between fasting triglycerides (FTG) level and atherosclerosis has not been consistent. However, triglycerides when measured in post prandial (PP) state emerge as independent risk factors for atherosclerosis that are stronger than HDL-C(134, 135).

Data from several studies including from our own institute as well as few others suggest that fasting triglyceride levels represent the residual TG burden which remains following the excess and more prolonged post prandial TG excursions that has been seen in insulin resistant and type 2 diabetic individuals. Furthermore, Karamanos group demonstrated that PPHTg is an independent phenomenon and could occur irrespectively of the fasting triglyceride concentrations, a clinical study carried out in healthy non diabetic subjects(34,121).

Results of the studies carried out in human as well as in animals have demonstrated that postprandial hypertriglyceridemia is an important risk factor in the development of cardiovascular disease(134,136,137).

To study the role of triglycerides in coronary artery disease, Patsch and co-workers studies post prandial lipaemia/hypertriglyceridemia in male patients with severe CAD and control subjects without CAD. Findings of this study suggested that post prandial triglycerides are independent predictors of CAD in multivariate analyses including HDL cholesterol. They also suggested that triglyceride metabolism is a critical determinant of cholesterol metabolic routing and also supported the concept that even the negative association between HDL cholesterol levels and CAD actually originates in part from a positive relation between CAD and plasma triglycerides(135).
Epidemiological, clinical and experimental studies that provide a basis that postprandial lipaemia is an important risk factor in the development of atherosclerosis and other cardiovascular disease\(^{(104,136,138,139)}\).

Postprandial lipaemia studies in human have shown a relationship between postprandial lipaemia/raised plasma remnant lipoprotein levels and increased carotid artery intima thickness in patients with familial combined hyperlipidemia and in patients with modestly increased cardiovascular risk\(^{(140,141)}\) and similar findings have been seen in a rabbit model of hereditary postprandial hypertriglyceridemia\(^{(142)}\).

Delayed clearance of TRLs has been linked with atherosclerosis progression in a number of clinical studies\(^{(104,135,137)}\). Recently a large scale clinical trial aimed to determine the association of triglyceride levels (fasting vs nonfasting) and risk of future cardiovascular events was carried out in healthy US women with a follow up of over 11 years by Bansal and group. Results of this study showed that postprandial triglyceride levels are associated with incident cardiovascular events, independent of traditional cardiac risk factors, levels of other lipids, and markers of insulin resistance. In contrast, fasting triglyceride levels showed little independent relationship with incident cardiovascular disease\(^{(143)}\).

One more prospective cohort study aimed to observe postprandial lipaemia and cardiovascular disease in general population (men and women) in Denmark with a follow up of over 25 years. Results of the trial showed that with increasing levels of postprandial triglycerides, levels of remnant lipoprotein cholesterol increased and these elevated nonfasting triglyceride levels are associated with increased incidence of cardiovascular disease and mortality both in men and women\(^{(144)}\).

Study carried out in type 2 diabetes patients with macrovascular disease have shown that diabetic patients display greaterer PPL particularly PP-hypertriglyceridemia, especially if they had macrovascular disease\(^{(145)}\).

PPL theory of atherosclerosis is also proven from \textit{in vitro} study where cytotoxic effect of hypertriglyceridemic serum and triglyceride-rich lipoprotein (TG-rich lipoprotein)
lipolyzed in vitro by purified lipoprotein lipase on cultured human umbilical vein endothelial cells (HUVECs) was studied. Results of this study showed that the interaction of endothelial cells with lipolytic remnants of TG-rich lipoprotein may play a role in the pathogenesis of atherosclerosis and that HDL may play an important role in inhibition of the endothelial cell injury produced by the lipolytic remnants of TG-rich lipoprotein\(^{146}\).

Results of study carried out to evaluate the relationship between levels of endothelial microparticles (EMPs, a sensitive indicator of endothelial disturbance) and post prandial lipaemia in healthy subjects without known cardiovascular risk showed that a significant elevation of plasma EMP levels in healthy, normolipidemic subjects. Also, plasma EMPs correlated with a postprandial hypertriglyceridemia and suggested that increase in EMPs may be a consequence of postprandial triglyceride-rich lipoproteins\(^{147}\).

**Lipoprotein Lipase**

Paul Hahn 1943, first realised the existence of LPL, when he observed that i.v. injection of heparin eliminates post prandial lipaemia following a fatty meal\(^{148}\). Since then, lot of advancement has been made in understanding of LPL’s function and structure. It is a water soluble enzyme, found in luminal surface of endothelial cells of capillaries and is widely distributed in adipose, heart, and skeletal muscle tissue, as well as in lactating mammary glands\(^{149,150,151}\). The best known function of LPL is hydrolysis of triglycerides into two FFA and one monoacylglycerol molecule for tissue utilization. However, it is also involved in promoting the cellular uptake of chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids\(^{152,153}\).

In addition to LPL’s central role in metabolism and transport of lipids, it has been found to be associated with obesity, insulin resistance, T2DM, Alzheimer’s disease, chylomicronaemia and atherosclerosis\(^{154}\).

Besides LPL’s central role in lipoprotein metabolism, it is thought to be involved in several other pathophysiological conditions, to name a few are hypertriglyceridemia, insulin resistance, diabetes, obesity and atherosclerosis. Decreased adipose tissue LPL
activity has been observed in animal models of diabetes and in patients with insulin resistance and diabetes. This adipose tissue LPL activity is known to increase in patients receiving anti diabetic therapy. The increased secretion of cytokines from adipose tissue in insulin resistance subjects also diminishes adipose tissue LPL activity. Therefore it appears that this is not plasma LPL activity which determines hypertriglyceridemia and in fact activity of adipose tissue lipase, hepatic lipase and plasma lipase collectively determines the fate of hypertriglyceridemia.

Visceral Fat and Subcutaneous Fat
Prevalence of obesity is increasing all over the world\(^{(1,155)}\). Two important component of obesity are visceral obesity and subcutaneous obesity that are associated with risk of diabetes and the metabolic syndrome.

Data suggest that SF is inherently different from VF and produces substances that can act systemically to improve glucose metabolism\(^{(156)}\) whereas, visceral adiposity is known to contribute to the development of insulin resistance, T2DM, hypertension, cerebrovascular and cardiovascular disease\(^{(157)}\). However, the exact mechanism by which visceral fat posses greater risk than subcutaneous fat is not clear. Some researchers have suggested that FFA released by adipose tissue, a primary event in the development of insulin resistance in animal models\(^{(158)}\) and secreted adipokines like IL-1, IL-6, TNF- \(\alpha\), resistin, reduced adiponectin may be behind the deleterious effect of visceral fat\(^{(159,160)}\).

Adipose Tissue and Adipokines
Adipose tissue has been the main organ for storage of energy, they also functions as an important endocrine organ. Adipocytes release a number of peptide hormones, cytokines and other biologically active molecules including tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-6 (IL-6), plasminogen-activator inhibitor-1 (PAI-1), angiotensinogen, leptin, adiponectin and resistin. When the amount of adipose tissue is incresed, as seen in obesity, the production of many of these secreted products is altered. Mohamed-Ali have also suggested that some of the adipokines may be involved in the development in insulin resistance associated with obesity\(^{(161)}\).
Evidence from knockout animal models suggests that the adipose tissue, in spite of being a minor site for glucose uptake in vivo, may play a major role in controlling overall glucose metabolism. Significant findings have been observed in transgenic mouse models where lack of white adipose tissue leads to severe insulin resistance, elevated lipid levels, and undetectable leptin levels and diabetes. In this model and in other mouse models with reduced adipose tissue, infusions of leptin, transgenic overexpression of leptin, or surgical implantation of white adipose tissue can reverse the diabetic phenotype and insulin resistance can be reversed by leptin replacement. Though, Considine has reported higher leptin levels in obese and insulin resistant humans and in subjects with a genetic predisposition for type 2 diabetes. Moreover, the recent discoveries of the adipocyte-secreted hormones resistin and adiponectin have received great attention and have been reported to modulate insulin sensitivity in mice, but the role of resistin in humans has been controversial and need further investigations.
Biomarkers of Atherosclerosis

Inflammation plays an important role in the progression of atherosclerosis and so as inflammatory markers and adhesion molecules. Among all inflammatory biomarkers, high-sensitivity C-reactive protein (hsCRP) has been most commonly used to predict the future risk of atherosclerotic complications. It is exclusively synthesized by the liver in response to factors released by macrophages and fat cells. hsCRP is found in blood, its level rises in response to inflammation. The physiological role of hsCRP is to bind to phosphocholine expressed on the surface of dead or dying cells in order to activate the complement system. hsCRP has been shown to predict the risk of cardiovascular disease even in healthy volunteers\(^{(171)}\).

Adhesion molecules intercellular cell adhesion molecule (ICAM 1) and vascular cell adhesion molecule (VCAM 1) has also emerged as an important biomarker of endothelial dysfunction, a primary event in the progression of atherosclerosis. These two adhesion molecules strongly participate on leukocyte adhesion to the endothelium. VCAM-1 is expressed on endothelium prone to develop atherosclerosis in some knock out model of mice fed on atherogenic diet\(^{(172)}\). ICAM-1 is expressed on the endothelium overlying atheromatous plaque in coronary and carotid arteries\(^{(172,173,174)}\). Since, atherosclerosis is being increasingly recognized a post prandial phenomenon, a recent clinical study was carried out by Diana and co-workers to investigate the association between postprandial triglycerides, insulin and glucose after ingestion of a standardized lipid-rich test meal, and soluble cellular adhesion molecules (CAM) in young healthy subjects. They classified subjects as either normal responders (NR) (postprandial triglyceride maxima < 260 mg/dl) or high responders (HR) (postprandial triglyceride maxima > 260 mg/dl) and compared levels of ICAM 1 AND VCAM 1 in HR and NR. Results of this study showed that Fasting ICAM-1 and VCAM-1 levels were significantly higher in HR as compared to NR. They also found significant correlation of VCAM-1 with postprandial triglycerides area under the curve\(^{(175)}\).
Thiazolidinediones

Thiazolidinediones (TZDs) also known as glitazones is a class of drugs known to improve insulin sensitivity. These drugs act by activating transcription factors “peroxisome proliferator-activated receptors (PPARs)”, a family of nuclear receptors involved in regulation of cellular differentiation, development and metabolism of carbohydrate, lipids and proteins. TZDs enhance target-tissue insulin sensitivity in vivo where they function as high-affinity ligands for the nuclear receptor PPAR-γ, which is particularly abundant in fat cells\(^{176}\).

TZDs’s role in improvement of dyslipidemia has also been shown in a large prospective study where TZDs supplement improved dyslipidaemia by altering fat storage and lipid metabolism\(^{177}\). TZDs have also shown increased lipolysis of VLDL by increasing LPL mass and decreasing plasma levels of apo C III. Further, pioglitazone, a drug of class TZDs has shown significant improvement in post prandial lipaemia and cardiovascular risk in insulin resistant smokers\(^{178}\).

Experimental study carried out by Collino and coworkers with the aim of investigating the hepatic signalling pathway activated by PPAR-γ activation in wistar rats maintained on a high-cholesterol fructose (HCF) diet for 15 weeks. These rats were treated with pioglitazone (3 mg/kg) orally for the last 4 weeks of this diet. Results of this study showed that the rats fed on HCF diet exhibited hyperlipidemia, hyperinsulinemia, impaired glucose tolerance and insulin resistance. Pioglitazone administration evoked a significant improvement in lipid metabolism and insulin responsiveness. However, diet-induced PPAR-γ expression was unaffected by the pioglitazone treatment in this study\(^{179}\).

Anti insulin resistance effect of pioglitazone has also been demonstrated in the experimental study carried out recently in male wistar rats who were fed on high fat diet and treated with pioglitzione for 12 weeks. Results of this study showed that pioglitazone treatment prevents insulin resistance in these rats and suggested that liver AdipoR2 mediated glucose uptake is important in the prophylactic effect on insulin resistance\(^{180}\).
Several other experimental as well as clinical studies have also shown similar effects of pioglitazone and have suggested that pioglitazone development of T2DM. Some of the clinical studies have also shown that treatment with pioglitazone also prevents conversion of IGT to diabetes.

**Statins (HMG CoA Reductase Inhibitors)**

Statins is a class of drugs that are known to inhibit HMG CoA reductase, a rate limiting enzyme in the production of endogenous cholesterol. Statins are well accepted drugs for treatment of dyslipidemia in patients with increased risk of cardiovascular disease. In last decade statin’s role has also been accessed with respect to the development of type 2 diabetes mellitus. However, data of these studies in this regard have been conflicting as some clinical trials and experimental studies suggest that stains improve insulin sensitivity/glycemic control, insulin resistance and reduces incidence of T2DM\(^{181, 182, 183, 184}\), whereas, other clinical trials show that lipophillic statins significantly increase fasting insulin and HbA1c levels that are consistant with insulin resistance and increased incidence of type 2 diabetes\(^{185,186,187,188,189,190}\). Some experimental studies also exist which show that atorvastatin reduces insulin secretion, precipitates insulin resistance and does not prevent new onset diabetes\(^{191}\).
Animal Models of Diabetes

Why Do We Use Animals for Studies?

Animal models have been used in research for over 100 year and use of these animal models has lead to numerous significant findings that were not possible in human beings. Wide spread use of animal models particularly mammals in biomedical research is in practice because of the following reasons;

1. Provocation of disease in humans is strictly prohibited because of the obvious ethical consideration
2. Almost similar homology with human diseases at physiological and genetic levels
3. For better understanding of disease mechanisms
4. Controlled environmental conditions
5. Short life span
6. Finding new drug targets
7. Screening of new chemical entities
8. Ease of manipulation - animals models are good targets for genetic studies (knockout models)
9. To carry out histopathology of different tissues
10. Over 100 year of experience
11. Cost Effective – low cost of production and rearing
12. Ease of availability

Selection of Animal Model

Each animal model exhibits few characteristics of human diabetes. There is not a single model that could mimic the evolution of human’s T2DM completely. Therefore, selection of animal model depends on scientific strategy of the investigators and profile of study. Rodent models have proven of great use in biomedical research but they have not been able to disclose the exact mechanism of type 2 diabetes. Non rodent species like dogs, pigs and non human primates are closer to humans in terms of pathophysiology of
T2DM. However, there are some limitations with the use of these animals like expensiveness, practical difficulties, extreme care and ethical considerations. A classic animal model of T2DM should exhibit following characteristics;

- Genetically predisposed
- Metabolic Syndrome
- IR → Hyperglycemia → β cell failure

However, all of these characteristics do not exist simultaneously in any of the rodent model.

**Classification of Animal Models of Diabetes**

Srinivasan has classified animal models broadly into five categories:

1. Spontaneous/genetically derived diabetic animals
2. Diet/Nutrition induced diabetic animals
3. Chemically induced diabetic animals
4. Surgical diabetic animals
5. Transgenic/knock-out diabetic animals

1. **Spontaneous Diabetic Animals** Example-ZDF, ZF, ob/ob mouse, Cohen diabetic rat,

   GK rat, Torri rat, non obese C57BL/6, R. Monkey.

The advantage of these animals is that they develop of diabetes spontaneously and origin of diabetes involves genetic factors. These animals exhibit characteristic features of typical type 2 diabetes. They are mostly inbred model and variability of data obtained is minimum. Small sample size is another important advantage.

The disadvantages of these animal models are that they are mostly of monogenic inheritance and development of diabetes is highly genetically determined. Mortality rate in these animals is high due to ketosis and they require insulin treatment in later stage for survival. Also, these models are not easily availability and expensive.
2. **Diet/Nutrition Induced Diabetic Animals** Example- P. obsess, C57BL/6J Spinny mouse.

These animals develop diabetes associated with obesity and toxicity of chemicals on other vital organs of body can be avoided. However, they require longer period of dietary treatment and does not develop frank diabetes. Therefore, it is not apposite for diabetic complications studies. Also, these models are not suitable for screening of antidiabetic agents.

3. **Chemically Induced Diabetic Animals** Example-GTG treated obese mice, Wistar adult rats, Swiss Albino mice

Chemically induced diabetes is because of selective loss of pancreatic β-cells and do not require insulin treatment in later stage of follow up. Low rate of ketosis and mortality has been reported in these animals. These animals are good for screening of antidiabetic agents. Also, they are cost effective and easily available. However, the major drawback with the use of these animals is that diabetes developed is similar to type 1 diabetes of humans and sometimes spontaneous regeneration of β-cells is seen resulting in reversal of diabetes. Chemicals exert toxic effects on other body organs also and variability of data produced is high.

4. **Surgical Diabetic Animals** Example- Partial pancreatectomized animals

No toxicity of chemical diabetogens on other body organs is reported and disease developed resembles human type 2 diabetes due to reduced β-cells mass. However, disadvantage of this type of animal model is that they show high mortality rate because of the involved surgical procedure and post operative care. Also, dissection of alpha cells along with β-cells and deficiency amylase enzyme is there.

5. **Transgenic/knockout Diabetic Animals** - animals lacking genes involved Insulin signaling/glucose transport
The biggest advantage of these animal models is that direct role/effect of a particular gene or gene mutation can be investigated. Therefore, genetic mechanisms involved in T2DM may be understood. The disadvantage of these animals is that they are highly sophisticated and expensive in production and rearing.

Recently, a new rat model of type 2 diabetes has been developed where Wistar rats/Sprague-Dawley rats are kept on high calorie diet (high sucrose and or high fat diet) that leads to the development of metabolic syndrome. After the development of metabolic syndrome, a mild dose of streptozotocin is given to induce partial \( \beta \)-cells destruction and consequently type 2 diabetes develops\(^{(48,193)}\).

The biggest advantage of this type of animal model is that it exhibits classic symptoms of type 2 diabetes viz obesity, dyslipidemia, insulin resistance and glucose intolerance over the period of time and a partial destruction of \( \beta \)-cells is induced to unmask type 2 diabetes. However, the disadvantage of this type of model is that they require longer period of follow up and are not genetically predisposed.

For our study/this type of study it was the best suited model as; (i) Wistar/SD rats are established models of sucrose induced insulin resistance and hypertriglyceridemia (ii) this model has earlier been used for post prandial hypertriglyceridemia study (iii) high sucrose diet leads to dyslipidemia, alters glucose metabolism (iv) this model can be used in long term study and does not require any drug/insulin intervention for survival (v) progression of diabetes is similar to that observed in human type 2 diabetes mellitus.