Chapter 01

Introduction
India is diabetic capital of the world housing 40 million cases of diabetes mellitus (Sarah Wild et al, 2004). The word diabetes is derived from a Greek word, meaning *siphon*, referring to the discharge of an excess quantity of urine; and mellitus is Latin for *honey*. Thus diabetes mellitus means the passage of large amounts of sweet urine. This is derived from the fact that excess glucose in the blood spills over into the urine, absorbing fluids along with it (KF Peterson et al, 2006).

It is a metabolic disorder of varying etiology, which leads to a variety of complications. Hyperglycemia (a disorder characterized by the presence of an excess of glucose in the blood and tissues of the body) is a common feature of these conditions (Geiss L et al, 1997).

The 1997 WHO report has shown that there is a marked increase in the number of people affected with diabetes and this trend is scheduled to grow in geometric proportions in the next couple of decades (George S Eisenbarth, 2009). The global prevalence of Diabetes, as reported by Sarah Wild, in Diabetes care, 2004 provides estimates for 2000 and projections for 2030 (Sarah Wild et al, 2004).

**Table 1.1** Report showing marked increase in diabetes (WHO)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NO OF PEOPLE AFFECTED (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WORLD</td>
</tr>
<tr>
<td>1995</td>
<td>124.7</td>
</tr>
<tr>
<td>2000</td>
<td>153.9</td>
</tr>
<tr>
<td>2025</td>
<td>299.1</td>
</tr>
<tr>
<td>2030</td>
<td>366.2</td>
</tr>
</tbody>
</table>

Unfortunately, the brunt of this increase will be borne by the developing countries. These countries will see more than a 200% increase in the number of diabetics, whilst the developed countries will have a relatively meager increase in numbers of around 45%. Even today, the prevalence, and incidence, in developing countries is significantly more than in many
developed countries and its presentation is also different than what is traditionally described in the developed countries (Indian Diabetic association).

In India, the crude prevalence rate of diabetes in urban areas is about 9% and that in rural areas has increased to around 3% of the total population an urban-rural population distribution of 70:30 (Indian Diabetic association (www.indian diabetic association.org).

Surveys as reported by Indian Diabetic association - have also shown that the prevalence of Impaired Glucose Tolerance (IGT) is also high. It has been reported that the prevalence of IGT is around 8.7% in urban and 7.9% in rural areas.

Recently, another study has shown that the prevalence rates for urban areas are around 6%, whilst the figures in the rural areas were found to be around 5% (Indian Diabetic association).

Given the observation that around 35% of those with IGT will develop full blown diabetes within five years, the sheer numbers of those with diabetes seems overwhelming. Diabetes Mellitus, as a whole, is becoming more common throughout the world with an increase of about 2.8% annually [Fig 1.1]

The cost of diabetes in US as incurred in 2007 was estimated to be $174 billion (with 17.5 million diabetic patients) Medical costs includes $27 billion for care to directly treat diabetes, $58 billion to treat the portion of diabetes-related chronic complications that are attributed to diabetes, and $31 billion in excess general medical costs. The cost for India with 40 million patients will be $ 400 billion approximately (Tim Dall et al, 2008). Yet diabetes happens to be the seventh most common cause of death in the developed world (Wadwa RP et al, 2009).

There are multiple pathways being pursued to “cure” this disease or at least dramatically ameliorate the burden it imposes on patients and their families (Wadwa RP et al, 2009). Continuous glucose monitoring is already improving the lives of many patients by providing “real time” information with alarms for hypo- and hyperglycemia (Tamborlane WV et
Multiple groups are now studying devices that will control insulin pumps, in particular turning off insulin delivery to prevent hypoglycemia (Tamborlane WV et al, 2009).

In developed countries, such devices will hopefully rapidly become the standard of care for patients with insulin-dependent diabetes.

Though many patients do not consider such mechanical devices, especially the current "first" generation of devices, as a true cure, these
therapies will set the bar in evaluating immunologic therapies considered for prevention of diabetes and β-cell replacement. Thus, the bar will be high and hopefully ever higher over the next decade. At present, pancreatic (long term) (Kandaswamy R et al, 2006) as well as islet transplantation (short term) (Nanji SA and Shapiro AM, 2006; Shapiro AM et al, 2006) can cure type 1 diabetes but, for most patients, with unacceptable risks associated with immune suppression. It is likely that autoimmunity, in addition to alloimmunity, limits the therapeutic potential of either of these forms of transplantation (Laughlin E et al, 2008).

1.1 Diabetes

Diabetes is a disorder of metabolism that affects the body’s ability to use digested food for growth and energy. Glucose is a simple sugar in the blood, which is normally necessary to provide energy to the cells in the body. Whilst in the absence of glucose, many cells and organs can use other substitutes such as ketone bodies for a while, important cells such as those of the brain, the red blood cells and kidneys can only use glucose.

For glucose to enter the cells insulin must be present. The body has a mechanism which closely regulates the level of glucose in the bloodstream so that the cells of the body can get their required supply at all times.

The body stores the excess of glucose in the form of glycogen in liver and muscles. The glycogen is mobilized during the shortage felt by the body important to maintain a constant blood-glucose level.

The regulation is achieved by two hormones (produced in the pancreas) that have antagonistic actions: insulin and glucagon. Although many other mechanisms do come into play, major role is played by these two hormones.
1.2 Pancreas

The pancreas, involved in two major processes in the body; digestion and regulation, is composed of two main parts, the exocrine and endocrine. The exocrine part (digestion) secretes substances into the intestinal tract which help in digestion of the eaten food. These include lipase, which helps to digest fat, and amylase that helps to digest starchy foods. It also releases 'bicarbonate of soda' alkali, to neutralize any stomach acid that may otherwise damage the lining of the gut. The exocrine pancreas is directly connected to the intestinal tract through the pancreatic duct Fig 1.2. On average the pancreas grows to a maximum length of between 12.5cm and 15cm and can vary in weight between 60g and 100g.

Fig 1.2 Anatomy of Pancreas

[Source: American Medical Association.]

The endocrine region is responsible for monitoring of blood glucose levels by the help of insulin, glucagon and other hormones.
On the surface of pancreas are present cells in form of islands, referred to as islets of Langerhans [In honor of Langerhan]. These cells are on the basis of staining are categorized as α, β and δ cells, α producing glucagon, β cells synthesize insulin and δ cells are concerned with production of somatostatin, growth inhibitory hormone (Fig 1.4).

Fig 1.3 Pancreas showing pancreatic & bile duct opening in duodenum.  
[Source: American Diabetic association]

Fig 1.4 Islets of langerhans.  
[Source: Indian Diabetic association]
Insulin is synthesized and secreted by the beta cells of the pancreatic islets, as a precursor called Proinsulin. As shown in the figure 1.5a, Proinsulin is proteolytically digested to Insulin a protein hormone that contains 51 amino acids and smaller C peptide. C peptide is eventually excreted. Fig 1.5b depicts the release of insulin after meals.

1.1 (a) Metabolic effects of Insulin

Insulin is required by almost all of the body’s cells but its major targets are liver cells, fat cells and muscle cells. For these cells, insulin does the following:

- Stimulates liver and muscle cells to store glucose in glycogen,
- Stimulates fat cells to form fats from fatty acids and glycerol,
- Stimulates liver and muscle cells to make proteins from amino acids,
- Inhibits the liver and kidney cells from making glucose from intermediate compounds of metabolic pathways (gluconeogenesis).
As such, insulin stores nutrients right after a meal by reducing the concentrations of glucose, fatty acids and amino acids in the bloodstream (Fig 1.6).

Glucagon, a peptide of 29 amino acids secreted by alpha cells of Langerhan has antagonistic effect to insulin. Both these hormones play a vital role in controlling blood-glucose levels (Mayo et al, 2003).

1.1 (b) Metabolic Effects of Glucagon

Glucagon acts on the same cells as insulin, but has the opposite effects:

- Stimulates the liver and muscles to break down stored glycogen (Glycogenolysis) and release the glucose
- Stimulates gluconeogenesis in the liver and kidneys
According to Kangduk Choi and Young-Bum Kim, 2010, a fundamental mechanism for the maintenance of glucose homeostasis is the rapid action of insulin to stimulate glucose uptake and metabolism in peripheral tissues. Skeletal muscle is the primary site of glucose disposal in the insulin-stimulated state (De Fronzo et al, 1997).

Resistance to the actions of insulin in skeletal muscle is a major pathogenic factor in type 2 or type 1 diabetes mellitus (Yki-Järvinen H et al, 1990; Petersen KF, 2002); it also contributes to the morbidity of obesity, and complicates poorly controlled type 1 diabetes (autoimmune) (Taniguchi CM et al, 2006).

The ability of insulin to increase glucose transport in skeletal muscle is elicited by the translocation of glucose transporter 4 (Glut4), the major insulin regulated glucose transporter, from intracellular vesicles to the plasma membrane and transverse tubules (Pedersen O et al, 1990).

In muscle of type 2 diabetic subjects, the expression of the Glut4 gene is normal, and impaired glucose uptake by insulin action most likely results from altered trafficking or impaired function of Glut4 (Dohm GL et al, 1991; Kim YB et al, 1999).

Because glucose transport in response to other stimuli that use different signaling pathways is normal in muscle of type 2 diabetic subjects, the resistance to insulin stimulation may be due to impaired insulin signal transduction (White MF et al, 1998; Sale EM et al, 2008; Farese RV et al, 2005).

The review of Kangduk Choi and Young-Bum Kim (2010) has summarized the updated information on insulin signaling over the past decade, with particular emphasis on the molecular mechanism of human insulin.
resistance, and also address the physiological role of the newly identified player of insulin action (Kangduk Choi et al, 2010).

1.3 (a) Insulin receptor signaling

Insulin signaling involves a cascade of events initiated by insulin binding to its cell surface receptor, followed by receptor autophosphorylation, and activation of receptor tyrosine kinases, which result in tyrosine phosphorylation of insulin receptor substrates (IRSs) including IRS1, IRS2, IRS3, IRS4, Gab1, and Shc (Bourdeau et al, 2005; Harley EA et al, 2003).

As shown in Fig 1.7 Binding of IRSs to the regulatory subunit of phosphoinositide 3-kinase (PI3K) via Src homology 2 (SH2) domains results in activation of PI3K, which phosphorylates membrane phospholipids and phosphatidylinositol 4,5-bisphosphate (PIP2) on the 3' position. This complex activates the 3-phosphoinositide-dependent protein kinases (PDK-1 and PDK-2) resulting in activation of Akt/protein kinase B (PKB) and atypical protein kinase C λ and ζ, (PKCλ/ζ), each of which are serine/threonine kinases (Vinciguerra M et al, 2006; Sasaoka T et al, 2006).

Activated Akt phosphorylates its 160 kDa substrate (AS160), which stimulates the translocation of insulin-mediated Glut4 from intracellular vesicles to the plasma membrane (Cheatham B et al, 1994). Moreover, activation of PKCλ/ζ is also involved in the regulation of Glut4 translocation in response to insulin (Le Marchand-Brustel Y et al, 1995).

However, the insulin receptor (IR) is also dephosphorylated and inactivated by protein tyrosine phosphatases (PTPs), which comprise an extensive family of proteins that exert negative effects on insulin action and glucose metabolism (Björnholm M et al, 1997; Christine Longuet 2008).
Thus, the physiological regulation of insulin action is controlled by the balance between phosphorylation and dephosphorylation [Fig 1.7]. Most importantly, the PI3K pathway is thought to be a key component of the insulin signaling cascade, which is necessary for the metabolic effects of insulin on glucose transport and Glu4 translocation (Angela Bononi et al, 2011).

![Fig 1.7 Insulin Signaling cascade.](Image)

Indeed, insulin-stimulated PI3K activity decreases in skeletal muscle of type 2 diabetic subjects, providing evidence for a defect in insulin signaling that could contribute to impaired Glut4 translocation and insulin resistance (Sano H et al, 2003).
1.3 (b) Glucagon receptor signaling

The glucagon receptor is a 62 kDa protein that is activated by glucagon and is a member of the class B G-protein coupled family of receptors. (Mayo, K.E et al, 2003).

![G-protein coupled signaling](image)

**Fig 1.8** G-protein coupled signaling.


cAMP is a second messenger and propagates the message for many signaling pathways. It accomplishes this by acting mostly on a single enzyme - protein kinase A (PKA), which has four subunits - two regulatory subunits (r) and two catalytic subunits (c). In the absence of cAMP, they form a complex as r$_2$c$_2$ that is inactive. cAMP binds to the r subunits, freeing the catalytic subunits, which become active (Fig 1.8).

The active PKA subunits, in turn, can catalyze phosphorylation of many different proteins. For example, enzymes of glycogen synthesis are turned off by phosphorylation, whereas enzymes of glycogen breakdown are turned on by phosphorylation (Wakelam, M.J et al, 1986)

Glucagon stimulated gluconeogenesis and glycogenolysis are transduced via a G protein coupled receptor (GPCR)
Glucagon action in the liver maintains normoglycaemia in the fasted state via activation of a Gs protein, leading to the stimulation of adenylate cyclase activity, cAMP production, Epac, Torc2, PKA, and CREB activation (Kimbell SR et al, 2004;).

Glucagon also increases intracellular calcium in a phospholipase C dependent manner (Aromataris, E.C et al, 2006) and activates AMPK (Koo, S.H et al, 2005) p38 MAPK (W Cao et al, 2005; J Chen et al, 1998) and JNK (Jelinek LJ et al, 1993), through mechanisms that remain incompletely understood. Epinephrine and nor epinephrine (Fig 1.10) share the GPCR with glucagon.
1.4 Symptoms

Fig 1.11 illustrates the more common symptoms of diabetes:

- Increased urination especially at night [Polyuria]
- Increased thirst [Polydepsia]
- Extreme tiredness.
- Unexplained weight loss.
- Genital itching or regular episodes of thrush.
- Delayed healing of cuts and wounds.
- Blurred vision.

Many people do not manifest typical signs or symptoms commonly associated with T2DM and therefore, all persons over the age of 25 years should undergo an annual test to rule out the presence of diabetes. The
glucose concentration in blood is indicative of the severity of glucose intolerance (Table 1.2).

**Table 1.2** Whole blood and plasma values of glucose concentration.

*Source: Indian Diabetic association.*

<table>
<thead>
<tr>
<th>Glucose Concentration mg/100ml (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Whole Blood</strong></td>
</tr>
<tr>
<td>Venous</td>
</tr>
<tr>
<td>Capillary</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
</tr>
<tr>
<td>Venous</td>
</tr>
<tr>
<td>Capillary</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus</strong></td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
</tr>
<tr>
<td>(\geq 110) (6.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 110) (6.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 126) (7 mmol/l)</td>
</tr>
<tr>
<td>(\geq 126) (7 mmol/l)</td>
</tr>
<tr>
<td><strong>2 hours post Glucose Load or both</strong></td>
</tr>
<tr>
<td>(\geq 180) (10.0 mmol/l)</td>
</tr>
<tr>
<td>(\geq 200) (11.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 200) (11.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 220) (12.2 mmol/l)</td>
</tr>
<tr>
<td><strong>Impaired Glucose Tolerance</strong></td>
</tr>
<tr>
<td><strong>Fasting (if measured)</strong></td>
</tr>
<tr>
<td>(&lt;110) (&lt;6.1 mmol/l)</td>
</tr>
<tr>
<td>(&lt;110) (&lt;6.1 mmol/l)</td>
</tr>
<tr>
<td>(&lt;126) (&lt;7 mmol/l)</td>
</tr>
<tr>
<td>(&lt;126) (&lt;7 mmol/l)</td>
</tr>
<tr>
<td><strong>2 hours post Glucose Load</strong></td>
</tr>
<tr>
<td>(\geq 120) &amp; (&lt;180) (\geq 6.7 mmol/l &amp; &lt; 10 mmol/l)</td>
</tr>
<tr>
<td>(\geq 140) &amp; (&lt;200) (\geq 7.8 mmol/l &amp; &lt; 11.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 140) &amp; (&lt;200) (\geq 7.8 mmol/l &amp; &lt; 11.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 160) &amp; (&lt;220) (\geq 8.9 mmol/l &amp; &lt; 12.2 mmol/l)</td>
</tr>
<tr>
<td><strong>Impaired Fasting Glycemia</strong></td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
</tr>
<tr>
<td>(\geq 100) &amp; (&lt;110) (\geq 5.6 mmol/l &amp; &lt; 6.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 100) &amp; (&lt;110) (\geq 5.6 mmol/l &amp; &lt; 6.1 mmol/l)</td>
</tr>
<tr>
<td>(\geq 110) &amp; (&lt;126) (\geq 6.1 mmol/l &amp; &lt; 7.0 mmol/l)</td>
</tr>
<tr>
<td>(\geq 110) &amp; (&lt;126) (\geq 6.1 mmol/l &amp; &lt; 7.0 mmol/l)</td>
</tr>
<tr>
<td><strong>2 hours PG (if measured)</strong></td>
</tr>
<tr>
<td>(&lt;120) (&lt;6.7 mmol/l)</td>
</tr>
<tr>
<td>(&lt;140) (&lt;7.8 mmol/l)</td>
</tr>
<tr>
<td>(&lt;140) (&lt;7.8 mmol/l)</td>
</tr>
<tr>
<td>(&lt;160) (&lt;8.9 mmol/l)</td>
</tr>
</tbody>
</table>
1.4 (a) Targets for control

All the target levels given below in table 1.3 are generalizations and individual targets should be established. Laxity may be allowed in elderly patients; certain conditions require a much tighter control, e.g. pregnancy and maculopathy.

**Table 1.3** Target values to be watched *(Indian Diabetic Association)*

<table>
<thead>
<tr>
<th>Target Values</th>
<th>mg/dl</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous Plasma Glucose (mg/100ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>80-110</td>
<td>4.5 - 6.1</td>
</tr>
<tr>
<td>2 hours postprandial</td>
<td>120-140</td>
<td>6.7 - 7.8</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (HbA1c) (Range 4-7)</td>
<td>&lt;7.5</td>
<td>&lt; 7.5</td>
</tr>
<tr>
<td>Blood pressure (mm/Hg)</td>
<td>&lt;130/85</td>
<td>&lt; 130/85</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>&lt; 180</td>
<td>&lt; 4.66</td>
</tr>
<tr>
<td>HDL-Cholesterol (males)</td>
<td>&gt; 40</td>
<td>&gt; 1.03</td>
</tr>
<tr>
<td>HDL-Cholesterol (females)</td>
<td>&gt; 50</td>
<td>&gt; 1.3</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>&lt; 100</td>
<td>&lt;2.6</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt; 150</td>
<td>&lt; 1.7</td>
</tr>
</tbody>
</table>

1.4 (b) Body Mass Index

Along with the biochemical parameters controlling weight also becomes essential (particularly in type II diabetics). The body mass index (BMI) is calculated using following formula:

\[
BMI = \frac{\text{Weight in Kg}}{\text{Height in meters}^2}
\]
> 23 = Overweight; > 27 = Obese (for people from the Indian subcontinent)

Care must be taken that the weight is not decreased below the lower limit, a BMI of 18.5 signifies low body weight.

### 1.5 Types of Diabetes

Diabetes can be grouped into three types (American Diabetic Association):

- **Type 1-** Juvenile DM [previously called insulin-dependent diabetes (IDDM)];
- **Type 2-** Maturity On-set DM [previously called non-insulin dependent diabetes (NIDDM)] and
- **Gestational Diabetes.**

#### 1.5 (a) Type 1 diabetes mellitus

Type 1 diabetes affects approximately 15% of all people with diabetes. It is rare in the first nine months of life and has peak incidences at 12, and between 20 and 35 years of age. It is caused by the destruction of the insulin-producing β cells of the pancreas, resulting in absolute deficiency of insulin.

Type 1 diabetes is associated with both devastating chronic complications and acute life-threatening ketoacidosis and hypoglycemia (Imagawa, A et al, 2000; Arslanian S, 2002) due to the impairment in the metabolism of lipid, carbohydrates and proteins.
Red: β cells (Producing insulin),

Green: α cells (Glucagon producers),

Blue: δ cells: (Synthesizing somatostain).

Fig 1.12 Islets of Langerhan [Fluorescent micrograph].
(Source: Rorcman, Diabetes, 2005, 5(4), 187-191.)

Type I results from autoimmune destruction of the pancreatic β-cell [Fig 1.13].

Fig 1.13 Type 1 Diabetes flow chart of events.
(Source: Indian Diabetic Association.)

At present, scientists do not know exactly what causes the body's immune system to attack the beta cells, but the researchers believe that autoimmune, genetic, and environmental factors, possibly viruses, are involved.
Though Type 1 diabetes develops most often in children and young adults, yet the disorder can appear at any age. Symptoms, usually develop over a short period, although beta cell destruction can begin years earlier, making the pancreas to produce very little or no insulin.

The resultant increase in blood sugar level triggers the body to counteract by reducing the amount of glucose reabsorption in the renal tubules and thus excreting an excess in the urine (Pinhas-Hamiel, O et al, 1996). Failure to treat and control the sugar level in the blood, a person can lapse into a life threatening diabetic coma, also known as diabetic ketoacidosis (Fig 1.14).

If type 1 diabetes goes un-diagnosed, and/or patients fail to manage their insulin administrations properly, the patient can either

1. Develop high blood glucose levels (hyperglycemia) which leads to increased fatigue and even long term organ damage.
2. Develop low blood glucose levels (hypoglycemia) which leads to seizures or episodes of unconsciousness.

Markers of immune destruction of the β-cell are present at the time of diagnosis in 90% of individuals and these include antibodies to the islet cell (ICAs), to glutamic acid decarboxylase (GAD), and to insulin (IAAs).

Younger individuals typically have a rapid rate of β-cell destruction and present with ketoacidosis, while adults often maintain sufficient insulin secretion to prevent ketoacidosis for many years. The more indolent adult-onset variety has been referred to as latent autoimmune diabetes in adults (LADA). Figure 1.15 shows the comparative differences in blood vessels of diabetic and non diabetic people, note the thickening of endothelium hallmark of type I diabetes.
1.5 (b) Type 2 diabetes mellitus

The Epidemic of the New Millennium, Type 2 diabetes is characterized by insulin resistance and, at least initially, a relative deficiency of insulin secretion. In absolute terms, the plasma insulin concentration (both fasting and meal-stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis (Figure 1.16). With time, however, there is progressive beta cell failure and absolute insulin deficiency ensues. In a minority of type II diabetic individuals, severe insulinopenia is present at the time of diagnosis and insulin sensitivity is normal or near normal.

Most individuals with type 2 diabetes exhibit intra (abdominal (visceral) obesity (fig 1.17), which is closely related to the presence of insulin resistance. In addition, hypertension, dyslipidemia (high triglyceride and low HDL-cholesterol levels; postprandial hyperlipidemia), and elevated PAI-1 levels often are present in these individuals. This clustering of abnormalities (as illustrated in fig 1.16) is referred to as the "insulin resistance syndrome" or the "metabolic syndrome." Because of these abnormalities, patients with type 2 diabetes are at increased risk of
developing macrovascular complications (myocardial infarction and stroke).

Type II diabetes has a strong genetic predisposition and is more common in minority ethnic groups, i.e. Mexican-Americans, Latinos, American Indians, Pacific Islanders, than in individuals of European ancestry. The genetic cause(s) of the common variety of type 2 diabetes is (are) not well defined and, at present, no specific genes have been identified in the pathogenesis of this common metabolic disorder (Rosenbloom et al, 1999).

What most people don't realize is that a proper balance of micronutrients is a critical part of reducing insulin resistance, which, if left unchecked, will erupt into full-blown adult-onset diabetes.
Ninety percent of type II diabetics are found to be deficient in magnesium. The correlation between magnesium deficiency and the onset of type II diabetes has been known since 1976, yet few physicians prescribe supplementation with this inexpensive mineral to their diabetic patients. In fact, magnesium deficiency is one of the most under-diagnosed electrolyte deficiencies in modern medicine.

Daily supplementation in the range of 400 milligrams has been shown to significantly improve insulin sensitivity. Individuals predisposed to adult-onset diabetes have also been shown to demonstrate deficiencies in several other important micronutrients, including: chromium, vanadium, zinc, and a host of important antioxidant complexes. As well, they often exhibit a deficiency in dietary protein and a general imbalance in their protein-to-carbohydrate ratio (Jerry L. Nadler, 2000).
This is likely due to dietary patterns that favor excessive intake of dietary fats and high-glycemic foods that break down quickly into simple sugars, causing a rapid spike in blood sugar levels.

Other nutritional factors that have been found to be effective in mitigating symptoms of adult-onset diabetes and its precursor, insulin resistance, include: vitamin E (α-tocopherol), vitamin C (ascorbate), vitamin K (phyloquinone), β-carotene (pro-vitamin A), α-lipoic acid, flaxseed oil (omega fatty acids), vitamin B3 (niacin and niacinamide), vitamin B6 (pyridoxine), vitamin B12 (cobalamin), biotin, manganese, copper, vanadium and zinc (MacWilliam Communications Inc. Type II Diabetes: The Epidemic of the New Millennium).

Chromium, in its most bioavailable form as chromium picolinate, is a particularly potent insulin sensitizer and a key component in the body's ability to tolerate and regulate blood sugars. Interestingly, 90 percent of the Indian population does not consume even the minimum recommended daily dose of this important mineral. All of these micronutrients are easily available and affordable through the daily use of a high quality, broad-spectrum nutritional supplement (MacWilliam Communications Inc. Type II Diabetes: The Epidemic of the New Millennium).

The pharmaceutically pure nutritional supplement will provide these ingredients in a balanced formulation, where each ingredient is provided in its most bioavailable form and in a dosage necessary for long-term optimal health. Prevention of the disease — before the damage is done — is first and foremost. Unfortunately, by the time most diabetic patients are diagnosed with the disease, it is already too late. We now know that much of the initial damage from high blood sugar levels occurs through the oxidation of fats to form toxic lipid peroxides (MacWilliam Communications Inc. Type II Diabetes: The Epidemic of the New Millennium).

Numerous recent studies show that vitamin E, a fat-soluble antioxidant, provides significant protection against this damage, caused by uncontrolled lipid peroxidation. Clinical trials have shown that
supplementation with vitamin E, alone, causes a marked improvement of insulin action and a reduction in blood sugar levels and oxidative stress. Reduction of blood sugars and prevention of lipid peroxidation provide added protection against the onset of heart disease, common in the diabetic patient (MacWilliam Communications Inc. Type II Diabetes: The Epidemic of the New Millennium).

The pre-clinical stage of type II diabetes, responds particularly well to weight loss and dietary change. Fats and sugars are powerful free radical protagonists, so it's important to reduce these in the diet and in the body.

The key is the combination of a low glycemic diet fortified with essential fatty acids, nutritional supplementation targeted to enhance insulin sensitivity and optimize antioxidant protection, and regular aerobic exercise. Clinical studies have reported that the normalization of glycemic control can prevent diabetic microangiopathies and possibly cardiovascular complications (Rosenbloom AL et al, 1999).

1.5 (c) Gestational diabetes mellitus (GDM)

Women who develop diabetes during the pregnancy are classified as having gestational diabetes.

**Table 1.4** Diagnosis of GDM with a 100 g glucose load (ADA):

<table>
<thead>
<tr>
<th>TIME</th>
<th>BLOOD GLUCOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>≥95 mg/dl (5.3 mmol/L)</td>
</tr>
<tr>
<td>1-h</td>
<td>≥180 mg/dl (10.0 mmol/L)</td>
</tr>
<tr>
<td>2-h</td>
<td>≥155 mg/dl (8.6 mmol/L)</td>
</tr>
<tr>
<td>3-h</td>
<td>≥140 mg/dl (7.8 mmol/L)</td>
</tr>
</tbody>
</table>
In most women who develop GDM, the disorder has its onset in the third trimester of pregnancy. At least 6 weeks after the pregnancy ends, the woman should receive an oral glucose tolerance test and be reclassified as having diabetes, normal glucose tolerance, impaired glucose tolerance, or impaired fasting glucose (Table 1.4).

**Fig1.17** Fluctuating blood sugar levels in correlation to insulin levels.

*Source: Suckale Solimena 2008 Frontiers in Bioscience*

Gestational diabetes complicates about 4% of all pregnancies. Clinical detection is important, since therapy will reduce prenatal morbidity and mortality. Risk assessment for GDM should occur at the first prenatal visit.
Women at high risk (positive family history, history of GDM, marked obesity, and high risk ethnic group) should be screened as soon as feasible. If the initial screening is negative, they should undergo retesting at 24-48 weeks. While those of average risk should have the initial screen performed at 24-48 weeks.

A fasting plasma glucose concentration greater than 126 mg/dl (7.0 mmol/l) or a postprandial glucose greater than 200 mg/dl (11.1 mmol/l) establishes the diagnosis of diabetes and obviates the need for more formal glucose tolerance testing. Women who require more formal testing should receive a 100 gram oral glucose load with plasma glucose levels determined at baseline, 1 hour, 2 hours, and 3 hours. Figure 1.18 exhibits the plasma concentration of blood glucose during 24 hours.

1.6 Etiological factors for occurrence of Diabetes

There are variety of uncommon and diverse types of diabetes which are caused by infections, drugs, exo and endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of diabetes are classified separately (Reardon W et al, 1992).

(a) Exocrinopathies

Damage of the pancreas must be extensive for diabetes to occur. The most common causes are pancreatitis, trauma, and carcinoma. Cystic fibrosis and hemochromatosis also have been associated with impaired insulin secretion (Reardon W et al, 1992).
(b) Endocrinopathies

Since growth hormone, cortisol, glucagon, and epinephrine increase hepatic glucose production and induce insulin resistance in peripheral (muscle) tissues, excess production of these hormones can cause or exacerbate underlying diabetes. Although the primary mechanism of action of these counter regulatory hormones is the induction of insulin resistance in muscle and liver, overt type 2 diabetes mellitus does not develop in the absence of beta cell failure (Reardon VV et al, 1992).

(c) Infections

A variety of infections have been etiologically related to the development of diabetes mellitus. Of these, the most clearly established is congenital rubella approximately 20% of infants who are infected with the rubella virus at birth developing autoimmune type II diabetes later in life. These individuals have the typical type 1 susceptibility genotype, DR3/DR4 (Reardon W et al, 1992).

Fig 1.18 Common complications associated with Diabetes.

Source: Indian Diabetic association.
Drugs

A large number of commonly used drugs have been shown to induce insulin resistance and/or impair beta cell function and can lead to the development of diabetes mellitus in susceptible individuals. Diabetes if not monitored and controlled can affect severely various other organs. Chronic hyperglycemia is associated with long term consequences that include damage and dysfunction of the cardiovascular system, eyes, kidneys and nerves (Fig 1.19).

1.7 Complications in Diabetes

The complications of diabetes are divided into two broad groups:

a. **microvascular**: neuropathy, nephropathy, retinopathy.

b. **macrovascular**: ischemic heart disease, stroke, peripheral vascular disease (Tamborlane et al, 2009, Garg SK, et al. 2007)

Together these make diabetes the seventh most common cause of death in the developed world (Wadwa RP et al, 2009).

1.7 (a) Neuropathy

The neuropathies are among the most common, most neglected and difficult to treat long-term complications of diabetes, affecting up to 50% of patients. (Andrew et al, 2004, Dyck et al, 1993, Young MJ et al, 1993 and Kumar. S et al, 1994).

It exists as two forms:

i. Somatic neuropathy (SN).

ii. Autonomic Neuropathy (AN).

Neuropathies are characterized by a progressive loss of nerve fibers, which may affect both principle divisions of the peripheral nervous system (SN) and autonomic nervous system (AN).
Both the neuropathies affect the whole body, and presents with diverse clinical pictures.

i. **Somatic neuropathy**

The most important outcome of somatic neuropathy is the development of diabetic foot followed by diabetic ulceration and possible amputation (Carrington AL et al, 2002).

Distal symmetrical polyneuropathy; chronic symmetrical symptoms affecting peripheral nerves with the longest nerves usually affected first; Sensory and motor functions affected in varying degrees, but may be predominantly sensory.

Often associated with neural dysfunction, Signs and symptoms vary commonly seen is tingling or numbness (with or without pain) pain usually bilateral beginning in the feet, spreading proximally in stocking. Later the upper extremities develop similar manifestations and progress upwards; Loss of balance, especially with the eyes closed, and painless injuries due to loss of sensation.

**Mononeuropathy multiplex:** An individual nerve can be affected, such as the peroneal nerve, resulting in foot drop, median neuropathy of the wrist, ulnar neuropathy of the elbow. Symptoms usually comprise similar to those described for SN; this might be in the form of solitary nerve involvement (American Diabetic Association Medical Dictionary A.D.A.M.http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001786/, 2010).

**Diabetic neuropathic cachexia:** presents with severe weight loss usually in older subjects often not diagnosed as having diabetes; followed by severe pain and signs and symptoms of SN; muscle weakness is rare (Micheal Knoop et al, 2008).

ii. **Autonomic Neuropathy (AN)**

Autonomic neuropathy is a nerve disorder that affects involuntary body functions, including heart rate, blood pressure, perspiration and digestion.
It isn't a specific disease. Autonomic neuropathy refers to damage to the autonomic nerves. This damage disrupts signals between the brain and portions of the autonomic nervous system, such as the heart, blood vessels and sweat glands. Diabetes is probably the commonest cause of autonomic neuropathy, and causes functional changes in many systems (Fig 1.20) including the cardiovascular, urogenital and gastrointestinal systems (John FB Morrison, 2001).

(b) Nephropathy

Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects ~40% of type 1 and type II diabetic patients. Incipient nephropathy is the stage of microalbuminuria; Albumin excretion is the most prominent symptom it can be estimated in 24 hour urine collection (Jorge et al, 2005).
End Stage Renal Disease

Renal replacement therapy (dialysis and/or renal transplant) is the treatment for end stage renal disease (ESRD).

Table 1.5 Nephropathy marker albumin in urine (Viberti et al, 1983 and Gall MA et al, 1993).

<table>
<thead>
<tr>
<th></th>
<th>24 hour collection</th>
<th>Timed collection</th>
<th>Spot collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg / 24 hours</td>
<td>ug / min</td>
<td>ug/mg Creatinine</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt; 30</td>
<td>&lt; 20</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30 – 300</td>
<td>20 - 200</td>
<td>30 - 300</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>&gt; 300</td>
<td>&gt; 200</td>
<td>&gt; 300</td>
</tr>
</tbody>
</table>

(c) Hypertension

Hypertension is a risk parameter in the diagnosis of the Diabetes (Tarnow L et al, 1994). A raised systolic blood pressure >/= 135 mm Hg and/or a raised diastolic blood pressure >/=85 mm Hg are the criteria leading to the diagnosis of hypertension.

Table 1.6 Blood pressure values in hypertension.

<table>
<thead>
<tr>
<th>Blood pressure stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre hypertension (120 to 139/80 to 89 mm Hg)</td>
</tr>
<tr>
<td>Stage 1 (140 to 159/90 to 99 mm Hg)</td>
</tr>
<tr>
<td>Stage 2 (&gt;160/&gt;=100 mm Hg)</td>
</tr>
</tbody>
</table>

(d) Obesity
Obesity is a risk factor for the development of insulin resistance, with pancreatic beta cells compensating for insulin resistance by augmenting insulin secretion (Critical organic report, 2009). 

Because it can take up to 10 years or longer for obese individuals to develop Type II diabetes, the full impact of the childhood obesity epidemic on the rate of Type 2 diabetes in young adults has not yet been seen (Critical organic report, 2009). 

Obesity, especially "central", "visceral", "truncal", "android" (i.e. abdominal) is a major (AHA/NHLBI) or sine qua non (IDF) criteria for the diagnosis of the Metabolic Syndrome (Eva Kassi et al, 2011).

(e) Atherosclerotic Cardiovascular Disease (ASCVD) 

The presence of the metabolic syndrome (pre clinical stage of diabetes) raises the lifetime risk for both ASCVD and type II diabetes mellitus (T2DM). Average relative risks are increased about twofold for ASCVD and fivefold for T2DM compared with those for individuals without the metabolic syndrome. Type II is more prone to ASCVD (Bhagat S Jaiswal, 2012, Scott M et al, 2005 and H Rober Superko and Spencer King III, 2008).

(f) Eye complications 

Diabetes mellitus is the most common cause of blindness amongst the 25-65 year age group (Report of the Working Group on Disease Burden for 12th Five Year Plan, 2011, Government of India). The major eye complications of Diabetes being: 

i. Glaucoma  

ii. Cataract  

iii. Retinopathy  

i. Glaucoma
It is a group of eye diseases that can lead to blindness by damaging the optic nerve. The eye continuously produces a fluid, called the aqueous, that must drain from the eye to maintain healthy eye pressure.

Patients do not experience any symptoms or early warning signs at the onset of glaucoma. This is why glaucoma is often called "the sneak thief of sight." (National Eye Institute, US).

![Normal Vision and Glaucoma Vision](image)

**Fig 1.20 Normal and Glaucoma vision.**

Source: National Eye Institute.

People with diabetes are 40% more likely to suffer from glaucoma than people without diabetes. The longer someone has had diabetes, the more common glaucoma is. Risk also increases with age. *(American Diabetes Association)*

Glaucoma occurs when pressure builds up in the eye. In most cases, the pressure causes drainage of the aqueous humor to slow down so that it builds up in the anterior chamber *(American Diabetes Association)*.

The pressure pinches the blood vessels that carry blood to the retina and optic nerve. Vision is gradually lost because the retina and nerve are damaged. There are several treatments for glaucoma. Some use drugs to reduce pressure in the eye, while others involve surgery *(American Diabetes Association)*.

**ii. Cataract**
A cataract is a progressive cloudiness, hardening, and yellowing of the normally transparent lens of the eye.

Non-diabetic also show the occurrence of cataracts, but people with diabetes are 60% more likely to develop this eye condition. Diabetics tend to get cataracts at a younger age and have them progress faster. (Research committee report of Baker IDI, 2008)

Nerve cells in the retina convert incoming light into electrical impulses. These electrical impulses are carried by the optic nerve to the brain, which finally interprets them as visual images. At birth, the natural lens is clear and very flexible. The lens becomes more rounded to focus on near objects and thinner (or stretched) to focus on objects that are far away.

This hardening and yellowing of the lens over time also causes the most common type of cataract, called a nuclear sclerotic cataract. "Nuclear" refers to the gradual clouding of the central portion of the lens, called the **nucleus**; "sclerotic" refers to the hardening, or **sclerosis**, of the lens nucleus (Research committee report of Baker IDI, 2008).

### iii. Retinopathy

Diabetic retinopathy is a general term for all disorders of the retina caused by diabetes, generally for several years. (Kangduk Choi et al, 2011)
Diabetic retinopathy is categorized into two types (Figure 1.21)

- Background
- Proliferative.

Retinopathy in diabetic subjects is basically a manifestation of small blood vessel disease and appears to be a function of the metabolic defect rather than the clinical type of diabetes.

Duncan et al, (1958) have stated that it complicates diabetes secondary to pancreatic disease. Furthermore, Waltman et al, (1979) and Liang et al, (1980) have demonstrated that the effects of diabetes on the retina can be delayed and perhaps even ameliorated if the blood glucose level is rigorously controlled.
(a) **Pathophysiology** - preliminary stage.

Cunha Vaz, (1978), Krupin T et al, (1980), and Klemen UM et al, (1980) have demonstrated that even before there is any ophthalmoscopic evidence of retinal disease there is functional circulatory disturbance. Foremost is an apparent breakdown of the blood-retinal barrier (Figure 1.22).

The blood vessels supplying blood to retina do not allow its escape from vessels, due to the presence of many encircling tight junction complexes between contiguous endothelial cells in the case of the intraretinal vessels termed as inner blood-retinal barrier and between cells of the retinal pigment epithelium referred to as outer blood-retinal barrier. When fluorescein is injected in systemic circulation of early diabetes the amount of fluorescein entering the vitreous is increased.

There are several hypothesis put forth for this leakage of fluorescein,

1. According to Cunha Vaz, (1978), this is because of increased permeability of the retinal vessels.

2. Tso et al, (1980) doctrine says it’s the fault in the pigment epithelium.

3. In support of the latter hypothesis, Klien et al, (1980) have demonstrated increase in vitreal fluorescein in guinea-pigs with streptozotocin induced diabetes, since that species lack intraretinal blood vessels.

4. However, there are reports of altered membrane permeability as studied by Kirber et al, (1980) and morphological changes in the pigment epithelium Wallow & Engerman, (1977) experiments support the increased vascular permeability concept by finding the penetration of the junctions by horse-radish peroxidase in alloxan-diabetic dogs.

In interpreting these findings caution is necessary, as irrefutable evidence of a defective blood-retinal barrier has not been ruled out. Klein et al, (1980) has demonstrated the fluorescent dye enters the vitreous from the aqueous fluid and not from retina.
This leakage can be ameliorated if normal blood glucose is maintained, suggesting that metabolic factors are of major pathogenetic importance. According to Kohner EM (1975) this possible breakdown in the blood-retinal barrier is accompanied by arteriolar dilatation and an increase in retinal blood flow and this in turn constitutes an auto regulatory response to tissue hypoxia and hyperglycemia as indicated by Hill and Artherton (1979).

The reason for tissue hypoxia may be altered metabolism or insulin dependent intraretinal processes (Hill & Atherton, 1979).

Elevated levels of glycosylated hemoglobin also contribute to tissue hypoxia during erythropoiesis. The elevated blood glucose, increases levels of glycosylated hemoglobin by the factor of two to three, which prevents the binding of 2, 3-diphosphoglycerate. This does not release the oxygen to tissue, leading to hypoxia. Though hypoxia is observed in retinopathy, yet correlation between glycosylated hemoglobin and diabetic retinopathy remains to be established (Alec Garner, 1981).

(b) Background retinopathy

Background retinopathy is progressively characterized by microaneurysms, intraretinal punctuate hemorrhages and yellow waxy serous exudates and, occasionally, cotton-wool spots secondary to acute ischemia. These abnormalities are mostly located to the posterior part of the retina

(i) Microaneurysms

Morphologically there are two types of microaneurysms. One type develops due to localized outpouching of the capillary wall, whilst the other

Fig 1.24: Normal and retinopathy vision.
Source: diabetic retinopathy,
mechanism for the formation of the first type of microaneurysms are due to weakness of the capillary, which in turn is caused by pericyte degeneration as observed by Cogan and his group (1961). Pope C H Jr, (1960) on the other hand states that it is because of the changes in the basement membrane, capillary obliteration to leave a residual stump, and abortive attempts at revascularization of areas of capillary closure.

The second type of microaneurysm appears to begin as enlargement of retina which is because limited hyperplasia causing both circumferential dilatation and longitudinal growth. Such elongation and widening predominates on the venous side of the retinal circulation and produces excessive tortuosity and kinking which, in some instances, appears to proceed to a loop-type of aneurysm as demonstrated by Ashton (1958).

According to Archer D B (1976), it is also possible that similar capillary hypertrophy underlies the bizarre intraretinal microvascular anomalies seen in relation to areas of retinal ischaemia, where they may represent limited attempts at neovascularization.

(ii) Yellow waxy serous exudates, edema and intraretinal punctuate hemorrhages

Thin-walled microaneurysms are the principal causes of yellow waxy exudates and intraretinal hemorrhage in the diabetic retina. Edema probably is the most serious of the manifestations of background retinopathy and if it involves in macular area, it is as a consequence of undue endothelial cell permeability. Another possibility is that oedema fluid stems from the adjacent choroidal circulation due to a breakdown in the blood-retinal barrier presented by the retinal pigment epithelium (Tso et al, 1980).

(iii) Cotton-wool spots

Occlusion of the central retinal artery, supplying the oxygen to the retina causes it to starve of oxygen leading to acute focal ischemia, which in turn
forms the cotton-wool spots. Kohner et al (1969) has reported that this further progresses to arteriolar necrosis of accelerated hypertension in diabetic subjects.

However the experimental work of Ashton et al, 1966 indicates the involvement of ischemic phenomena affecting the nerve fiber layer of the retina. He has further demonstrated that the swelling of the individual axons in the hypoxic area is due in part to intracellular edema and in part to failure of axoplasmic transport as researched by McLeod and his group, 1977.

A new approach of multimedia mapping methods enables to differentiate between three diabetic retinopathy phenotypes, allowing personalized management strategies (Gabriele E Lang, 2007). High-resolution imaging by optical coherence tomography provides additional, new information about morphological findings in diabetic retinopathy (Gabriele E Lang, 2007).

In a study carried out by Fritsche P et al, 2002 three different phenotypes were again clearly identified after an initial 2-year follow-up period. The discriminative markers of these phenotypes were: microaneurysm formation rate, measurements of fluorescein leakage, and signs of capillary closure in the capillaries surrounding the FAZ (Table 1.8)

**Table 1.7 Different patterns and phenotypes of retinopathy**

*(Gabriele E Lang, 2007)*

<table>
<thead>
<tr>
<th>VF/RLA</th>
<th>Red dot formation rate</th>
<th>FA</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern A (62%)</td>
<td>4 ng/ml</td>
<td>3/year</td>
<td>normal</td>
</tr>
<tr>
<td>Pattern B (20%)</td>
<td>4 ng/ml</td>
<td>3/year</td>
<td>normal</td>
</tr>
<tr>
<td>Pattern C (18%)</td>
<td>4 ng/ml</td>
<td>3/year</td>
<td>abnormal</td>
</tr>
</tbody>
</table>

(iv) Underlying vascular changes

(a) Arterioles

The retinal arterioles undergo thinning and become transparent. The process continues without any symptoms, there is leakage of plasma through abnormally permeable endothelial lining. This leakage may be enhanced by the increased transmural pressure gradient associated with the arteriolar dilatation characteristic of early diabetic retinopathy as reported by Skovborg et al, (1969). Ashton (1953) has reported that occlusion of the terminal arterioles may be responsible for the areas of capillary closure.

Precisely how the blockage of blood vessels is brought about is not clear since the thinning of blood vessel cannot cause leakage of such degree so as to completely obliterate the lumen. Other factors such as increased blood viscosity and platelet abnormalities are also involved. McMillan (1976) has reported, increase in blood viscosity in patients with diabetic microangiopathy, causative factors including raised levels of acute phase proteins such as fibrinogen and β globulin.

Paulsen & Koury (1976) have reported that it is due to possibly, reduced erythrocyte malleability attributable to the binding of excessive amounts of HbA1c to the cell membrane.

Alexander W D et al, (1979) has demonstrated the role of increased viscosity in retinopathy which develops in early diabetic state after severe trauma involving haemoconcentration. Dobbie reasons undue stickiness of the circulating platelets in diabetes as a major cause, (Dobbie J G et al, 1973).

Bloodworth, Toussaint & Dustin (1963) have demonstrated basement membrane thickening.

Vracko R (1974) has shown increase in collagenous tissue is also probable reason for retinopathy.

Cogan et al (1961) hypothesized that pericyte (smooth muscle cells of arterioles) degeneration is the major cause of retinopathy.
(B) Venules:

Skovborg et al (1969) demonstrated mild venular dilatation, as a probable reason in the early stages. It can be reversed controlling the metabolic state (Larson HW, 1960). An earlier view that it is caused by venous stasis is difficult to sustain in the light of fluorescein angiographic studies showing increased blood flow in background retinopathy (Alec Garner, 1981).

(c) Proliferative retinopathy

In Proliferative diabetic retinopathy, there is arteriolar and capillary changes, these blood vessels undergo thinning and leak. The leakage causes decrease in the blood flow (Cunha Vaz JG et al, 1978).

Kohner, Dollery and Cunha Vaz have also reported that the blood flow eventually may even drop below normal (Kohner EM, 1975), and it is at this stage that neovascularization takes place from previously dilated and hyperplastic vessels on the venous side of the circulation. While Archer DB (1976) has demonstrated a limited degree of revascularization of the ischemic areas.

Because of the continuous leakage from the new vessels, the gel structure of the vitreous body collapses causing it to wrinkle and eventually detach from the retina, as reported by Davis MD (1965), Constable I (1975).

According to Kloti R (1967) retinal ischemia disposing to the neovascularization is responsible for the vitreous detachment. Taniguchi et al (1973) reports that the newly formed blood vessels do not have properly developed tight junctions, and are fenestrated therefore they tend to leak.

Wise and Ashton have hypothesized, that the leaky blood vessels liberate an angiogenic factor which, because of a capacity to diffuse into and accumulate in the vitreous space, stimulates endothelial proliferation on the retinal surface (Ashton N, 1957).

Attempts to demonstrate the proliferation of endothelium the chick Chorioallantoic membrane (Glaser BM et al, 1980) and cornea (Kissun &
Garner, 1977) were used as assay substrates. The studies revealed angiogenic activity in extracts of retina from normal neonate animals.

There is a lot yet to be revealed as far as the pathophysiology of diabetic retinopathy is concerned much of the present attention is being directed, appropriately to an understanding of the initial events since these seem to be potentially reversible.

A lot depends on determining the extent to which permeability of the blood-retinal barrier increases as a primary event and the mechanisms involved therein. Information is also accruing of metabolically controlled events in the circulating blood and considerable effort is now being applied in an effort to put the various pieces of the jigsaw together (Alec Garner, 1980).

1.7 Mechanisms leading to diabetic retinopathy

The micro- and macro-vascular complications of diabetes are the most common causes of renal failure, blindness and amputations leading to significant mortality, morbidity and poor quality of life however, incomplete understanding of the causes of diabetic complications hinders the development of mechanism-based therapies (Eva LF et al, 2004).

*In vivo* and *in vitro* experiments implicate a number of enzymatic and non-enzymatic metabolic pathways in the initiation and progression of diabetic complications (Brownlee M, 2004)

The different pathways involved are as follows:

1. Increased Polyol pathway activity leading to sorbitol and fructose accumulation, NAD(P)-redox imbalances and changes in signal transduction; (Eva LF et al, 2004; Vincent AM et al, 2004)

2. Non-enzymatic glycation of proteins yielding "advanced glycation end-products" (AGEs); (Vincent AM, 2004)
(3) activation of protein kinase C (PKC), initiating a cascade of intracellular stress responses (Ishii H, 1998); and

(4) increased hexosamine pathway flux (Kaneto H et al, 2001).

Only recently has a link among these pathways been established that provides a unified mechanism of tissue damage. Each of these pathways directly and indirectly leads to overproduction of reactive oxygen species (ROS) (Leinninger G et al, 2004; Yorek MA, 2003).

1.7 (a) Polyol pathway

The polyol pathway is a two-step metabolic pathway in which glucose is reduced to sorbitol, which is then converted to fructose (Fig 1.24). It is one of the most attractive mechanisms to explain, at least in part, the cellular toxicity of diabetic hyperglycemia (Vincent AM et al, 2004) because

(i) It becomes active when intracellular glucose concentrations are elevated,

(ii) The two enzymes aldose reductase and glutathione reductase are present in human tissues and organs that are sites of diabetic complications, and

(iii) The products of the pathway and the altered balance of cofactors generate the types of cellular stress that occur at the sites of diabetic complications.

Inhibition (or ablation) of aldose reductase, the first and rate-limiting enzyme in the pathway, reproducibly prevent diabetic retinopathy in diabetic rodent models, but the results of a major clinical trial have failed to show similar results. The spectrum of abnormalities known to occur in human diabetic retinopathy has enlarged to include glial and neuronal abnormalities, which in experimental animals are mediated by the polyol pathway (Eva LF et al, 2004).
The endothelial cells of human retinal vessels have been noted to have aldose reductase (Fig 1.24). Specific polymorphisms in the promoter region of the aldose reductase gene have been found associated with susceptibility or progression of diabetic retinopathy (Vincent AM, 2004).

![Fig 1.25: The polyl Pathway and Diabetic retinopathy. Source: R. Paul Robertson, DIABETES, VOL. 52, MARCH 2003](image)

1.7 (b) Advanced Glycation End Products

In diabetics the excess of glucose causes an increase flux through the glycolytic pathway (and polyol pathway) which leads to increases in dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) both 3-carbon phosphorylated sugars which get converted to methyglyoxal (MG), MG is a marker of AGEs and is very reactive. Oxidative stress leads to its accumulation in diabetics (Brownlee M, 2004; Vincent AM, 2004).
Calorie restriction is thought to interfere with this system, the lack of calories decreases the NADH/NAD+ ratio. NAD+ is required for GAPDH to function, thus by increasing availability the removal of both G3P and DHAP is increased and its subsequent production of MG is also decreased (Vincent AM, 2004).

In over nutrition (Type II diabetes) NAD+ is not available for GAPDH. Available G3P and DHAP get converted to MG and thus oxidative stress. Diabetics suffer from another problem and that is the constant hyperglycemia which leads to the extracellular glycation (Hipkiss AR, 2010).

Hyperglycemia increases the glycation process, and is especially apparent in insulin independent tissues such as red blood cells, peripheral nerve tissue cells, endothelial cells, eye lens cells, and kidney cells (Claudia et al, 2010).
**Fig 1.27:** Schematic diagram of formation of AGE.
*Source: Namiki, Ann Reviews in Biochem (2007)*

**Fig 1.28:** Wolff pathway showing formation of Amadori products.
*Source: Journal of Medicine Volume 247 June 2008*
Outside of cells glycation doesn't occur due to the production of reactive carbonyls through glycolysis, instead there are other pathways by which the damaging components can be reached, e.g. Namiki pathway, Wolff pathway illustrate the formation of Amadori products Fig 1.27.

1.7 (c) Protein kinase C (PKC) Pathway

![Image of PKC pathway]

**Fig 1.29:** Activation of protein kinase C (PKC), initiating a cascade of intracellular stress responses.

*Source: Diabetes, 1998*

According to Khan ZA, exposure of cultured endothelial cells to high levels of glucose leads to rapid induction of a number of protein kinase family members (Khan ZA et al, 2005; Xin X et al, 2004; Khan ZA et al, 2005) suggesting that these proteins may play a role in transducing the adverse effects of hyperglycemia in the retinal vasculature.

Excellent review article by Zia A Khan and S Chakrabarti (2006) has explained the activation of an important protein kinase, PKC (Ishii H et al, 1998) in the context of diabetes (Khan ZA et al, 2005). PKC isoforms which show significant activation in animal models of chronic diabetes include PKCa, βI, βII, γ, and δ (Vincent AM et al, 2004). However, PKCβI and II show the highest level of induction in the retina as well as the heart.
and aorta of the diabetic animals (Idris I et al, 2001; Inoguchi et al, 1992; Shiba T et al, 1993). The mechanism of PKC activation (Fig 1.28) may involve increased diacylglycerol (DAG) levels which have been shown in the retina of diabetic animals (Xia P et al, 1994) and vascular cells exposed to high glucose levels (John A Hanover et al, 2010).

1.7 (d) Hexosamine pathway influx

According to John A Hanover l et al (2010) organisms have evolved a

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**Fig 1.30:** Enzymes that mediate hexosamine pathway.

A robust network of signaling pathways allows them to distinguish sources of food from pathogens (immunity), regulate the uptake and utilization of food (metabolism), and adapt to nutrient availability (gene expression) (Love Dc et al, 2005).

One of the most evolutionarily ancient of these pathways is the nutrient-sensing addition of O-GlcNAc to target proteins (Hart G W et al, 2007; Ross MD et al, 2000; Banerjee S et al, 2009; Hanover JA et al, 2001). Many reviews have appeared in the past few years detailing many aspects of O-GlcNAc metabolism (Kamemura K et al, 2003; Vosseller K et al, 2001; Whelan SA et al, 2006). In addition, several excellent reviews have focused on methods of detection of O-GlcNAc (Zachara NE, 2009).

### 1.8 Oxidative stress

Increased production of reactive oxygen species (ROS) and reactive nitrogen (RNS) and subsequent oxidative stress are the major factors responsible for diabetic complications in target organs species, both in type 1 and type II diabetes.

According to Joseph L Evans, 2003 elevated glucose, and possibly FFA levels contribute to the pathophysiology of diabetes via the generation of ROS and consequent activation of numerous stress sensitive pathways (Feldman EL et al, 1998).

The causative link among hyperglycemia, mitochondrial ROS generation, oxidative stress, and the development of diabetic complications has been already suggested (Joseph L et al, 2003).

ROS (and RNS), by inflicting macromolecular damage, may play a key direct role in the pathogenesis of diabetes. ROS also function as signaling molecules (analogous to second messengers) to activate several stress sensitive pathways (indirect role) (Rosen P et al, 2001).

In addition, in type 2 diabetes, there is growing evidence that activation of stress-sensitive pathways, such as NF-kB, p38 MAPK, JNK/SAPK, and hexosamine, by elevations in glucose and possibly FFA levels leads to
both insulin resistance and impaired insulin secretion (Russell JW et al, 1999; Schmeichel AM et al, 2003; Apfel SC, 1999; Tomlinson et al, 1997; Mohamed et al 1999).

Thus ROS and oxidative stress, induced by elevations in glucose and possibly FFA levels, may play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways.

In the enzymatic mechanisms are included, superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and nitric oxide synthase enzymes, among others. On the contrary, in the non-enzymatic
mechanisms are comprised of antioxidants and trapping agents such as ascorbic acid, α-tocopherol, β-carotene, glutathione, flavonoids, uric acid, cysteine, vitamin K, serum albumin, bilirubin, and trace elements as zinc and selenium, among others (Mohamed AK et al, 1999).

Whenever the natural antioxidant mechanism in mammals under some circumstances can be inefficient, a dietary intake of antioxidant compounds becomes an alternative, it has been suggested that there is an inverse relationship between dietary intake of antioxidants and the incidence of diseases caused by the deficiency on these substances (Gonçalves C et al, 2005).

According to Oscar M Mosquera et al, 2007 in recent years, synthetic antioxidants such as buthylated hydroxyanisole (BHA) and buthylated hydroxytoluene (BHT) are added to food preparations because they are good free radical scavengers, even though there are some experimental evidences that they induce DNA damage (Bassman JH et al, 2004; Oscar MM, 2007).
Therefore, there is an increasing interest in searching antioxidants from natural origin to scavenge oxidative stress induced free radicals to prevent human body from oxidative stress produced by ROS and RNS species (Kovacic. P et al, 2001).

According to Hostettmann and Terreaux, 2000, the estimated number of higher plant species in the world is of 400,000, the fact that plant secondary metabolites are characterized by an enormous chemical diversity and that currently one-fourth of all prescribed pharmaceuticals compounds in developed countries are directly or indirectly (semi-synthetic) derived from plants (Hostettmann K, Terreaux C 2000).

As plants produce a huge amount of antioxidants they can represent a source of new compounds with antioxidant activities (Cuendet M et al, 2000 Bassman JH, 2004).

### 1.9 Antioxidants

ROS and RNS are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies, among others (Kovacic. P et al, 2001; Haliwell B et al, 1999).

The ROS and RNS may be effectively neutralized, by enhancing the cellular defenses, in form of antioxidants.

The generation of ROS begins with rapid uptake of oxygen and the activation of NADPH oxidase and production of the superoxide free radical (O$_2^-$) (Halliwell B et al, 1999).

\[ 2O_2 + \text{NADPH} \xrightarrow{\text{OXIDASE}} 2O_2^- + \text{NADP}^+ + H^+ \]
Superoxide is then rapidly converted to hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD). (Miller ER et al, 2005).

\[ 2 \text{O}_2^- + 2 \text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2 \]

In presence of chloride ion, which is ubiquitous, hydrogen peroxide is converted to hypochlorous acid (HOCl), a potent oxidant. The reaction is catalysed by myeloperoxidase (MPO) (Valko M et al, 2005).

\[ \text{Cl}^- + \text{H}_2\text{O}_2 + \text{H}^+ \xrightarrow{\text{MPO}} \text{HOCl} + \text{H}_2\text{O} \]

The MPO independent mechanism, though not as important as previous one, is still essential. ROS are also generated from superoxide and hydrogen peroxide via oxidative burst by Fenton (A) and Haber-Weiss (B) reaction (Cadenas E et al, 2000).

(A) \[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot \text{OH} + \text{OH}^- + \text{Fe}^{3+} \]

(B) \[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot \text{OH} + \text{OH}^- + \text{O}_2 \]

Reactive nitrogen species are also important. The free radical nitric oxide (NO⁺), first described as endothelium derived relaxation factor (EDRF), is produced from arginine by nitric oxide synthase (NOS) (Klatt, P et al, 2000).

\[ \text{L- Arginine} + \text{O}_2 + \text{NADPH} \xrightarrow{\text{NOS}} \text{NO} + \text{Citrulline.} \]

An inducible nitric acid synthase (iNOS) is capable of continuously producing large amount of NO⁺, which is 'cytotoxic killer molecule'.

Although the direct toxicity of NO⁺ is modest, it gets greatly enhanced when it reacts with superoxide to form peroxynitrite, a very strong oxidant.

\[ \text{NO}^- + \text{O}_2^- \rightarrow \text{ONOO}^- \]
Peroxynitrite can react with aromatic amino acid residue to form nitrotyrosine, which can lead to enzyme inhibition.

Antioxidant acts as scavengers of these free radicals. The scavenging reaction between free radical (FR•) and an antioxidant (H-A) can be written as:

\[ \text{FR}^{•} + \text{H-A} \rightarrow \text{FR-H} + \text{A}. \]

Antioxidants react with FR•, which is a stable free radical and is reduced to the FR-H. Thus antioxidant relieves the oxidative stress by decreasing the free radical content of the cells (Halliwell B et al, 1999).

### 1.10 Flavonoids

Flavonoids are Key components to reduce Oxidative stress thus prevent Diabetic retinopathy.

Flavonoids are a group of polyphenolic compounds, which have the diphenylpropane (C6-C3-C6) skeleton, ubiquitously found in fruits, and vegetables (Harborne JB et al et al, 2000).

The flavonoid family includes flavones, flavonols, flavanones, flavanurons, flavans, flavonols, leucoanthocyanidins, anthocyanidins, aurones, chalcones, and isoflavones. The structural difference in each flavonoid family results from the variation in the number and arrangement of the hydroxyl groups and the extent of glycosylation (Rice Evans CA et al, 1996).

Epidemiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart disease. In addition, the flavonoids exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, antiradical, and antioxidant actions (Wang H et al, 1997).
Especially, they may exert antioxidative effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal ion chelators, properties attributed to the phenolic hydroxyl groups attached to the ring structures (Toma’s-Barbera’n et al, 2000; Warner D et al, 2004).

In diabetic retinopathy, the cellular components, especially the blood vascular system of retina which is rich in polyunsaturated fatty acids, proteins, enzymes and nucleic acid are susceptible to oxidative attack resulting in highly damaging lipid peroxidation, protein peroxidation and DNA damage (Hung Hc et al 2004, Franco Oh et al, 2004). For example - Genistein is a potent and specific in vitro inhibitor of tyrosine kinase action in the autophosphorylation of the epidermal growth factor (EGF) receptor (Orlidge A et al, 1987, Canfield A et al, 1990; Hellstrom M et al, 2001).

The peroxidation products formed are also highly cytotoxic. Prevention of lipid peroxidation, protein peroxidation and DNA damage by use of medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations.

1.11 Pericytes

Cells of great importance in pathophysiology of Diabetic retinopathy. loss of retinal pericytes is one of the first histological features of diabetic retinopathy. (Cogan D et al, 1961).

Retinal pericytes are smooth muscle-like cells with attenuated processes enveloping the abluminal surface of microvessels and sharing a common basement membrane with the underlying endothelium (Diaz-Flores et al, 1991).

Pericytes provide vascular stability, exert control over endothelial cell proliferation, morphology, and microvessel architecture. Pericytes express α smooth muscle actin (α-SMA) and have been implicated to have a contractile function, thus regulating blood flow. They are proposed to regulate microvascular angiogenesis and synthesize components of the vascular basement membrane (Orlidge A et al, 1987, Canfield A et al, 1990; Hellstrom M et al, 2001).