Literature Review
1 Epidemiology

Type 2 diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90% of cases globally. This deadly disease has taken the form of an epidemic in both developing and developed countries with increasing number of people suffering from diabetes. From 366 million in 2011, the number of diabetics is expected to reach a level of 552 million in 2030, at an average annual growth of 1.7 times the growth of total adult world population, making diabetes one of the most serious public health problems worldwide. Ninety percent of the diabetic patients are either obese or overweight with maximum number of patients falling in the age ranging from 40-60 years, also most of the diabetes deaths occur in low and middle income countries including India. In the present scenario India has 160 million diabetics and is expected to have the largest number of diabetic patients by 2025. In the last two decades, type 2 diabetes, once thought to be a metabolic disorder exclusively of adulthood, has become increasingly frequent in obese adolescents. For example, in Japan (H.Sekikawa et al, 2000) 80% of all new cases of type 2 diabetes diagnosed are in children and adolescents. Likewise, in Taiwan (P. Chou et al, 1994) 54.2% of new cases were diagnosed with type 2 diabetes, with an incidence of 6.5% per 100,000. In contrast, in U.K. the minimum incidence of type 2 diabetes in children (<17 years of age) was 0.53 % 100,000−1 year−1 (N.G. Forouhi et al, 2006). According to Diabetes Atlas 2010, India has 40.9 million diabetics and this number is likely to become 87 million by 2030, making India the “diabetes capital of the world” (J.E. Shaw et al, 2010).

Since, the number of diabetics are increasing at an alarming rate in our country and worldwide, it is of due importance to understand the underlying factors helping in the progression of this disease and to develop a mechanistic approach towards finding a solution of this growing epidemic. By developing clinical methods to lower the incidence and to increase the recovery of individuals diagnosed with type 2 diabetes mellitus, it will soon be possible to control the incidence, progression and clinical manifestation of this rapidly rising syndrome.

Type 2 diabetes is thought to occur in genetically predisposed individuals who are exposed to a series of environmental influences that finally precipitates the onset of this clinical disease. However, there are various other factors responsible for the development of type 2 diabetes mellitus:
Genetic Factors

Genetic Markers
Family History
Thrifty Genes

Demographic Characteristics

Sex
Age
Ethnicity

Behavioral And Lifestyle Related Risk Factors

Obesity (including distribution and duration)
Physical inactivity
Diet
Stress

Metabolic Determinants And Intermediate-Risk Categories Of Type 2 Diabetes

Impaired glucose tolerance
Insulin Resistance
Pregnancy related determinants
  • Parity
  • Gestational diabetes
  • Diabetes in offspring of women with diabetes in pregnancy
  • Intrauterine malnutrition or overnutrition

Table 1. Factors responsible for type 2 diabetes mellitus.
2 Pathogenesis

The pathogenesis of type 2 diabetes mellitus (T2DM) is complex and involves the interaction of genetic and environmental factors. A number of environmental factors have been shown to play a role in the development of the disease, particularly excessive caloric intake leading to obesity and a sedentary lifestyle. Patients with T2DM consistently demonstrate three cardinal abnormalities: defective insulin secretion, particularly in response to a glucose stimulus (β-cell dysfunction); resistance to action of insulin in the peripheral tissues, particularly muscle and fat but also liver (insulin resistance); and increased glucose production by the liver (hyperglycemia). The common forms of T2DM are polygenic in nature and are due to a combination of insulin resistance and abnormal insulin secretion. From a pathophysiological standpoint, it is actually the inability of the pancreatic beta cell to adapt to the reductions in insulin sensitivity that occurs over the lifetime of human subjects, that precipitates the onset of T2DM (S. Fajans et al., 2001). The most common factors that place an increased secretory burden on the beta cell are puberty, pregnancy, a sedentary lifestyle, and an over-eating leading to weight gain.

In Type 2 diabetes mellitus, insulin is present at normal levels but the tissues do not respond to the hormone. This is caused by decreased sensitivity of target tissues to the metabolic effect of insulin. In type 2 or non-insulin-dependent diabetes mellitus, muscle and fat cells become "resistant" to the actions of insulin and compensatory mechanisms that are activated in the β-cell to secrete more insulin are not sufficient to maintain blood glucose levels within a normal physiological range (E.A. Davis et al., 1999). The precise way these factors interact, finally lead to the onset of T2DM.

2.1 Obesity

Obesity is one of the major risk factors and an underlying cause of type 2 diabetes mellitus. Therefore most of the diabetic individuals have a problem of central adiposity. In case of obesity there occurs β cell compensation such that there is increased insulin secretion even in response to normal glucose levels. This abnormality can only be possible if there is increased β-cell sensitivity in response to glucose. The root cause of this defect can be: (a) an increase in β cell mass and thus presence of more GLUT2 receptors, as found in obese individuals or (b) increased
expression of hexokinase as compared to glucokinase leading to increased insulin secretion in response to wide range of glucose concentrations, thus resulting in hyperinsulinemia. Central obesity can also alter the levels of secretion of adipokines which regulate and mediate processes like lipid metabolism, lipolysis, and insulin mediated glucose uptake and utilization. The abnormal decrease in the levels of these hormones adds to the already existing conditions for T2DM. In obesity, the levels of adipocyte hormones like leptin and adiponectin are decreased while the levels of TNF-α and resistin are found to be increased thus making obesity a cause for T2DM. (P.A. Kern et al, 2001)

Adiponectin is a secreted protein of 247 amino acids, produced specifically from adipocytes. (P.E. Scherer et al, 1995) In circulation, adiponectin is found in two forms: a full length protein and a globular form (due to proteolytic cleavage). Both these forms have different modes and sites of action. The globular form acts mainly on skeletal muscles and increases the β-oxidation of fatty acids via activating PPARα, along with AMPK mediated increase in GLUT4 translocation. Whereas, the full length protein functions only to decrease the hepatic glucose output by activation of AMPK (adenosine monophosphate kinase) in liver. Since the increase in triglyceride content as in cases of obesity, interferes with the insulin mediated phosphorylation of PI-3 kinase, thereby affecting insulin action, both, full length protein and globular form of adiponectin mediate their insulin sensitizing actions by decreasing the triglyceride content. (T. Yamauchi et al, 2003)

Another probable mechanism by which adiponectin helps in reducing diabetes related complications, is by reducing the levels of inflammatory cytokines like TNF-α and IL-6. Adiponectin and these cytokines especially TNF-α antagonize each other and are negatively correlated. Adiponectin suppresses TNFα-induced inflammatory changes in endothelial cells by blocking inhibitory nuclear factor-κB phosphorylation and nuclear factor-κB activation without affecting TNFα-mediated activation of c-Jun N-terminal kinase, p38, and Akt.
Fig. 1 Adiponectin can activate AMPK and PPARα in the liver and skeletal muscle. In skeletal muscle, both globular and full-length adiponectin activate AMPK, thereby stimulating phosphorylation of ACC, fatty-acid oxidation, and glucose uptake. Adiponectin activates PPARα thereby also stimulating fatty-acid oxidation and decreasing tissue TG content. In the liver, only full-length adiponectin activates AMPK, thereby reducing molecules involved in gluconeogenesis and increasing phosphorylation of ACC and fatty-acid oxidation. Adiponectin activates PPARα, thereby stimulating fatty-acid oxidation and decreasing tissue TG content. These alterations increase insulin sensitivity in vivo. WAT, White adipose tissue; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase. (Adapted from Kadowaki and Yamauchi /Endocrine Reviews; 2005)

The signaling pathway by which adiposity is linked to insulin resistance is however, complex. It is mediated by more than one factors, like non-esterified fatty acids (NEFA), tumor necrosis factor α (TNF-α) and hormones like leptin and adiponectin. These modulate insulin action in the peripheral tissues contributing to insulin resistance. (M. Stumvoll, 2002; Steven Kahn et al, 2006)
Fig 1.2 Genes responsible for obesity and insulin resistance interact with environmental factors (increased fat/caloric intake and decreased physical activity), resulting in the development of obesity and insulin resistance. (adapted from Steven Kahn/ nature 444; 2006)

2.2 Insulin resistance

Insulin resistance indicates the presence of an impaired biologic response to either exogenously administered or endogenously secreted insulin. It is manifested by decreased insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscles and by impaired suppression of hepatic glucose output. (K. Hida et al, 2005)

Understanding of the mechanism and signaling of insulin action allows to divide the insulin resistance into three types: prereceptor, receptor and postreceptor insulin resistance. Type A insulin resistance is due to a decrease in the number of insulin receptors, whereas patients with type B insulin resistance have circulating autoantibodies against some portion of their insulin receptors, and this results in impaired binding of insulin to target cells (Vandana Saini, 2010). The majority of insulin resistance is of the postreceptor type due to the extraordinary complexity of the intracellular pathways of insulin action.
Table 1.2 Various types of Insulin Resistance in type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Insulin Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Pre-Receptor Resistance</td>
</tr>
<tr>
<td>• Antibodies against insulin.</td>
</tr>
<tr>
<td>• Mutant insulin structures.</td>
</tr>
<tr>
<td>• Defect in primary structure of insulin β chain at one or more positions.</td>
</tr>
<tr>
<td>• Familial hyperproinsulinemia.</td>
</tr>
<tr>
<td>• B-C proinsulin: mutation at the cleavage site between the B chain and the connecting (C) peptide.</td>
</tr>
<tr>
<td>• A-C proinsulin: mutation at the cleavage site between the A chain and connecting (C) peptide.</td>
</tr>
<tr>
<td>ii. Receptor Resistance</td>
</tr>
<tr>
<td>• Type A. Decrease in insulin receptor number and/or affinity for the hormone.</td>
</tr>
<tr>
<td>• Impaired expression of receptor tyrosine kinase activity</td>
</tr>
<tr>
<td>• Point mutation blocks insertion of mature receptor into plasma membrane.</td>
</tr>
<tr>
<td>• Type B. Receptor blocked by circulatory antibodies to the receptor</td>
</tr>
<tr>
<td>• Leprechaunism: An autosomal recessively inherited disorder of insulin function that leads to severe intrauterine growth retardation, characteristic dysmorphic features and a disturbed glucose homeostasis. The defect underlying this disease is in structure of insulin receptor.</td>
</tr>
<tr>
<td>iii. Post-Receptor Resistance</td>
</tr>
<tr>
<td>• Decreased capacity of pancreatic β cells to compensate for the underlying insulin resistance by increased insulin secretion</td>
</tr>
<tr>
<td>• Possible under expression (down regulation) of β-cells glucose transporters (therefore failure to recognize and respond to hyperglycemia)</td>
</tr>
<tr>
<td>• Auto-antibodies to GLUT-2 transporter in β cells</td>
</tr>
</tbody>
</table>
2.3 Peripheral insulin resistance:

2.3.1 Skeletal muscle insulin resistance

1) Non Esterified Fatty Acids

NEFA's being the most critical of all; compete with glucose for substrate oxidation in isolated muscle. *(Barry Goldstein, 2002)* The increase in fatty acid metabolism leads to an increase in intramitochondrial acetyl coenzyme A (COA)/COA and reduced adenine dinucleotide (NADH)/NAD+ ratios, with subsequent inhibition of pyruvate dehydrogenase. This results in increased intracellular mitochondrial and cytosolic citrate concentrations result in allosteric inhibition of phosphofructokinase, the key regulating enzyme of glycolysis. Subsequent accumulation of glucose 6 phosphate would then inhibit hexokinase II activity, resulting in an increased intracellular glucose concentration and decreased glucose uptake finally leading to insulin resistance. *(G. Boden, I. Gerald Shulman, 2003)* Increased NEFA concentration inside the cells can also trigger a serine/threonine signaling cascade via fatty acid metabolites like diacylglycerol (DAG) and fatty acyl coenzyme A(fatty acyl COA). This pathway once activated causes phosphorylation of insulin receptor substrate 1(IRS1) and insulin receptor substrate 2(IRS2) thereby decreasing their ability to bind phosphatidylinositol-3-OH kinase (PI(3)K), this interaction is necessary for glucose transporter 4(GLUT4) translocation and insulin mediated glucose uptake. *(P.J. Randle et al, 1963)*

2) Triglyceride content

Glucose uptake and intramuscular triglyceride content has been found to follow an inverse relationship. The increase in the triglyceride content occurs due to imbalance of free fatty acid uptake and oxidation, as in case of obese individuals. The major reason for this can be the presence abdominal adiposity, since abdominal fat is lipolytically more active leading to increased circulating levels of NEFAs. This follows the muscular uptake of free fatty acids which is more than it can oxidize. The mechanism involved in this mismatch of uptake and oxidation is mediated by malonyl CoA. *(J.D. McGarry, 1995)* The increased insulin levels in circulation due to β cell dysfunctions mediate initial increase in glucose uptake by
skeletal muscles. This glucose entering the glycolytic pathway causes an increase in citrate levels by activating tricarboxylic acid (TCA) cycle and providing an alternate substrate for acetyl CoA carboxylase (ACC). ACC after activation generates malonyl CoA thus inhibiting carnitine palmitoyltransferase-1 (CPT-1) and blocking the transfer of fatty acyl CoA into mitochondria for oxidation (J.D. McGarry, 1995; S.T. Swanson, 1998; N.B. Ruderman, 1999) This accumulation of triglycerides like long chain fatty acyl CoA and diacylglycerol after activation sets several protein kinases into action, leading to serine/threonine phosphorylation of insulin receptor and resulting in decreased IR kinase activity finally causing insulin resistance. (Iria Nieto-Vazquez et al, 2008)

2.4 Hepatic glucose production: it's role in insulin resistance.

Liver is the main site for glucose synthesis and storage in the form of glycogen which can later be utilized for giving energy to carry out various cellular processes. The process of conversion of glycogen into glucose and glucose synthesis from other precursors is strictly controlled by the pancreatic hormone, insulin. (E. Stephen Borst, 2004; R. Gas, 2000) Insulin can inhibit both glycogenolysis and gluconeogenesis by activating and de-activating enzymes involved in these pathways. Insulin suppresses glycogenolysis by increasing phosphodiesterase activity and by changing the assembly of protein phosphatase complexes. (C.B. Newgard, 2000; D. Yeagley, 2001) Gluconeogenesis however is controlled by insulin mediated inhibition of phosphoenolpyruvate carboxykinase transcriptional activation via phosphorylation of foxhead transcription factor (Foxo1 and FoxoA2). (J.G. Jackson et al, 2000; R.K. Hall et al, 2000)

An alternate pathway of insulin mediated inhibition of glucose synthesis in liver is mediated via glucagon and free fatty acids. Insulin causes a decrease in glucagon secretion from pancreatic α cells thereby inactivating and downregulating both glycogenolysis and gluconeogenesis. (C. Wolfrum et al, 2004) Insulin also exhibits its anti-lipolytic effect by decreasing the activity of triacylglycerol lipase and other enzymes in adipocytes. This leads to decreased amount of fatty acids in circulation and decreased uptake by liver eventually resulting in reduction in gluconeogenesis and hence glucose production.
In case of obese individuals, insulin mediated inhibition of glucose production is impaired due to the presence of abdominal fat deposits. These deposits are resistant to the anti-lipolytic actions of insulin so insulin in unable to inhibit lipoprotein lipase activity resulting in increased levels of free fatty acids in circulation, thus more FFA are available to act as precursors for glucose synthesis in hepatocytes. (I. Magnusson et al, 1992; M.W. Schwartz et al, 2000)

2.5 β-cell dysfunction

The endocrine pancreas contains β-cells that secrete insulin in response to elevated glucose levels. Once secreted, insulin performs the function of maintaining normal glucose levels in the body by interacting with the plasma lemma receptors of a number of different cell types. It is the inability of the β cells to secrete required amounts of insulin in response to circulating levels of glucose, that paves the way for T2DM. The exact mechanism by which glucose stimulates insulin release from β cells is mediated by facilitated diffusion of glucose inside pancreatic β cells via glucose transporter GLUT2. This is present on the surface of pancreatic β cells. The numbers of these transporters are however found to be reduced on the cells of T2DM patients. Glucose after entering β cells is converted to glucose-6-phosphate, by one of the glycolytic, rate determining enzyme, glucokinase or hexokinase IV. (J.C. Bruning et al, 2000) Glucokinase acts as a glucose sensor of the β cells and leads to increased insulin secretion. (F.M. Matschinsky et al, 1998) The entry of glucose to β cells follows insulin release. The dose-response relationship leads to more insulin secretion as more and more glucose enters the pancreatic β-cell. The pathway by which glycolysis mediates insulin secretion depends upon the opening and closing of ATP dependent K+ channels present inside the cell. The step involving the oxidation of glyceraldehyde-3-phosphate generates NADH which eventually generates ATP. Increase in ATP concentration in β cells leads to blockade of ATP-dependent K+ channel causing membrane depolarization followed by increased cytosolic Ca2+ concentration. This causes fusion of preformed insulin granules with the plasma membrane and thus insulin secretion. (I.D. Dukes et al, 1994)
3 **Insulin signaling abnormalities in insulin resistance**

3.1 **Insulin signaling**

Insulin signaling is initiated through binding to and activation of its cell surface receptor that initiates a cascade of phosphorylation events, second messenger generation, and protein-protein interactions. This results in the diverse metabolic events in nearly every tissue. The insulin receptor consists of two insulin binding α subunits and two catalytic β subunits that are disulfide linked into an α2β2 heterotetrameric complex. (A. Pick et al, 1998) Insulin binds to the extracellular α subunits, activating the intracellular tyrosine kinase domain of β subunit. Once receptor β subunit phosphorylates its partner on specific tyrosine residues, it causes stimulation and intramolecular association of signaling molecules such as Shc and Grb, members of the insulin receptor substrate family (IRS1,2,3,4), Shc adaptor protein isoforms, and SIRP (signal regulatory protein) family members. (S. Obici, 2002)

The insulin receptor β subunit has also been shown to undergo serine/threonine phosphorylation, which might decrease the ability of receptor to autophosphorylate, as in case of obesity. The NEFA’s that enter the peripheral tissues change into lipid metabolites like fatty acyl CoA’s and diacylglycerol (DAG). DAG once formed activates novel PKC isoforms that catalyze the serine/threonine phosphorylation of the β subunit and thus attenuates insulin signaling, making way for insulin resistance. The activities of a number of protein kinase C (PKC) isoforms that catalyze the serine or threonine phosphorylation of the insulin receptor are found to be elevated in animal models of insulin resistance as well as in human subjects having insulin resistance. (J.K. Elmquist, 2003)

4 **Role of central nervous system in Type 2 Diabetes Mellitus**

Central nervous system plays an important role in insulin secretion and glucose homeostasis. Insulin has its receptors not only on peripheral tissues but also on the surface of brain cells where it maintains energy balance. (W. Fan, 2000) Deletion of these receptors from neurons has been found to induce obesity, (J.R. Zierath, 2000)
thereby highlighting their probable importance in pathogenesis of T2DM. Any alteration in the number of brain insulin receptors causes an imbalance in the glucose homeostasis, not leaving behind the liver glucose production. \textit{(J.Avruch et al, 1998)} These findings, clearly suggest that T2DM can also result due to underlying defects in central nervous system.

5 Oxidative stress

The term oxidative stress can be defined as a cellular condition when the production of reactive oxygen species (ROS) like superoxide anions, hydrogen peroxides etc. is greater than the quenching capacity of the cellular antioxidant enzymes and cellular reductants. \textit{(A.G. Danielsen, 1995)} In this condition the cell is under stress due to overproduction of ROS which react and cause oxidation of cellular biomolecules, resulting in peroxidation of the membrane lipids, affecting the cellular integrity, oxidation of all the cellular proteins including enzymes involved in metabolism as well as DNA oxidation leading to strand breaks and degradation, eventually resulting in cellular and tissue damage. \textit{(Peter Proctor, 1989)} In normal cells there exists a mechanism for controlling oxidative stress involving enzymes and other molecules. These enzymes include superoxide dismutase, catalase and glutathione peroxidase with GSH (L-γ-glutamyl-L- cysteinyl-glycine) as the major reducing molecule in the cell.

5.1 Superoxide dismutase (SOD)

Superoxide dismutase belongs to a class of enzymes that converts superoxide anions to molecular oxygen and hydrogen peroxide, quenching the harmful reactive oxygen species (superoxide \(O_2^-\)) and rendering it ineffective. SOD can be classified into two groups based on their reactive centres; Cu-Zn SOD and Mn SOD. MnSOD is found in mitochondria whereas Cu-Zn SOD is present in the cytosol. The mechanism by which SOD performs dismutation of superoxide ions can be summarized in two equations:

- \[ M^{(n+1)+} - \text{SOD} + O_2^- \rightarrow M^{n+} - \text{SOD} + O_2 \]
- \[ M^{n+} - \text{SOD} + O_2^- + 2H^+ \rightarrow M^{(n+1)+} - \text{SOD} + H_2O_2. \]

Where \( M \) is the metal ion at the reaction center or active site of the enzyme.
If $M = Cu (n=1); Mn (n=2); Fe (n=2); Ni (n=2)$.

5.2 Catalase

Catalase enzyme is majorly located in peroxisomes but is also found in mitochondria though at lower levels. The main function of this enzyme is to convert hydrogen peroxide ($H_2O_2$) to oxygen and water. This scavenging reaction is carried out in a two-step process:

$$H_2O_2 + Fe (III)-E \rightarrow H_2O + O=Fe (IV)-E (. +)$$

$$H_2O_2 + O=Fe (IV)-E (. +) \rightarrow H_2O + Fe (III)-E + O_2$$

Here $Fe (III/IV)-E$ represents the iron center of the heme group attached to the enzyme. $Fe (IV)-E (. +)$ is a mesomorphic form of $Fe (V)-E$, meaning the iron is not completely oxidized to $+V$, but receives some "supporting electron" from the heme ligand. This heme has to be drawn then as a radical cation ($.+$)

Hydrogen peroxide once generated as a result of several metabolic processes can severely damage cells and tissues thus, catalase converts these $H_2O_2$ to stable molecules ($O_2$ and $H_2O$) protecting the cell from free radical induced damage.

5.3 Glutathione Peroxidase

Glutathione peroxidase is the major hydrogen peroxide scavenger enzyme found both in cytosol and mitochondria. It reduces the lipid peroxides to their corresponding alcohols and hydrogen peroxide to water. This enzyme plays its anti-oxidant role in a single step process, in which reduced glutathione (GSH) is converted to oxidized glutathione (GSSG).

$$2GSH + H_2O_2 \rightarrow GS-SG + 2H_2O$$

After the oxidation, GSSG is converted back to its reduced form by the enzyme glutathione reductase, thus completing the cycle and regenerating the substrate for the action of glutathione peroxidase.
5.4 **GSH (L-γ-glutamyl-L-cysteinyl-glycine)**

GSH is the most abundant non protein thiol source and antioxidant in the cell. Apart from playing its antioxidant role, it also performs many other functions:

- GSH provides a substrate for GSH peroxidase.
- GSH conjugates with harmful exogenous and endogenous toxic compounds
- It reduces disulfide linkage of proteins and enzymes thus maintaining them in reduced state for their proper physiological action.
- This thiol source helps in maintaining the redox status of the cell.
- GSH also plays an important role in reduction of ribonucleotides to deoxyribonucleotides.

6 **Hyperglycemia induced oxidative stress**

In normal cells, when glucose is metabolized via tricarboxylic acid cycle (TCA), it produces two reducing powers; NADH and FADH₂. These electron donors transfer their electrons to complex I and complex III of the mitochondrial electron transfer chain (ETC) respectively. During this whole process, protons are driven out of the cell membrane through complex I, III and IV generating a potential gradient inside the cell for the synthesis of ATP via ATP synthase. However, in diabetic cells, the condition is almost reversed. Due to hyperglycemia, more glucose enters the TCA cycle generating an increased concentration of reducing equivalents (NADH and FADH₂). This increase pushes these electron donors more and more into the ETC, increasing the transfer of protons outside thereby leading to enhanced voltage gradient across the mitochondrial membrane. This rise in voltage gradient when reaches the critical threshold, blocks further transfer of electrons to complex III. The electrons thereby return to coenzyme Q, which transfers one electron at time to water, thus generating superoxide radicals. *(M.D Evans, M.S. Cooke, 2004)* These radicals increase the production of lipid peroxides by reacting with membrane lipids and also cause increase in the levels of H₂O₂, finally increase the total concentration of ROS inside the cell. *(Michael Brownlee, 2005)*
6.1 Activation of major damaging pathways

Hyperglycemia induced superoxide radical generation activates four major pathways of cellular damage. The exact mechanism is mediated via the inhibition of enzyme glyceraldehyde 3 phosphate dehydrogenase (GAPDH) of the glycolytic pathway. (S.S. Korshunov, 1991) Superoxide radicals cause DNA damage and thus activate the enzyme poly ADP-ribose polymerase (PARP), which on sensing the damage done, breaks NAD⁺ to nicotinic acid and ADP-ribose. PARP then polymerizes ADP-ribose units which get attached to several nuclear proteins including GAPDH (GAPDH is found in cytosol but it actually shunts in and out of nucleus where it helps in DNA repair) thereby reducing its activity. (X. Du et al, 2003; A. Sawa et al, 1997) This decrease in activity causes accumulation of glycolytic metabolites upstream of GAPDH activating four major pathways:

- Glyceraldehyde-3-phosphate accumulation activates advanced glycation end product (AGE) formation via major intercellular precursor methylglyoxal and also activates protein kinase C (PKC) pathway by forming diacylglycerol. PKC stimulation has been found to activate NAD(P)H oxidase in diabetes. Once activated NAD(P)H transfers electron from NAD(P)H to molecular oxygen, generating superoxide and thus adding to oxidative stress.

- Fructose-6-phosphate upon accumulation increases the flux through hexosamine pathway leading to the formation of UDP-N-acetylglucosamine (UDP-GlcNAc) via glucosamine-6-phosphate. Glucosamine-6-phosphate once formed can inhibit the activity of enzyme glucose-6-phosphate dehydrogenase, which is also involved in reduction of NADP⁺ to NADPH. This decreases the cellular ratios of NADP⁺/NADPH and thus causing oxidative stress either by decreasing the regeneration of GSH from GSSG or by decreasing the activity of antioxidant enzyme.

- Finally, increased amount of glucose gets directed into the polyol pathway converting glucose into sorbitol and finally fructose, utilizing NADPH and reducing its concentration inside the cell. Since NADPH is a substrate for glutathione reductase (GR), the depletion in NADPH levels decreases the activity of GR and an increase in free radicals thereby causing oxidative stress.
7 **Vitamins as potential anti-diabetic agents**

Vitamins are natural substances found in plants and animals and are known as essential nutrients for human beings. These are the components of a balanced diet which the human body cannot synthesize on its own (except vitamin D and vitamin K) and thus have to be consumed through diet either in the form of food or as supplements. The term vitamin is derived from words “vital” and “amine” because vitamins are required for life and were originally thought to be amines, however not all vitamins are amines. Vitamins are organic compounds required by humans in small amounts. An organic compound is considered vitamin if a lack of that compound in diet results in overt symptoms of deficiency. Since people suffering from T2DM are also found to be deficient in many vitamins this deficiency can add to many other causes of developing type 2 diabetes. Diabetes as a disease does not cause severe health problems, but the complications associated with diabetes can lead to problems. Since vitamins play an important role in glucose metabolism, understanding the impact of vitamin deficiencies and the potential utility of supplementation is relevant to the prevention and management of type 2 diabetes mellitus (T2DM).
7.1 Vitamin D

Vitamin D is an assemblage of fat-soluble secosteroids, i.e., steroids in which one of the bonds in the steroid rings is broken. (H.D. Schmidt, 2001) The two major physiologically pertinent forms are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). The structural dissimilarity between vitamin D2 and vitamin D3 is in their side chains. The side chain of D2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24. These are collectively known as calciferol. Vitamin D2 (made from ergosterol) is produced by invertebrates, fungus and plants in presence to UV light and is not produced by vertebrates. Vitamin D3, on the other hand, is produced photo-chemically in the skin from 7-dehydrocholesterol. 7-dehydrocholesterol is produced in relatively large quantities in the skin of most vertebrate animals, including humans.

Vitamin D3 is synthesized in the skin of vertebrates after exposure to UV-B light and also by intake of wide variety of foods.

Foods containing Vitamin D include catfish - 85gm provides 425 IU, mackerel-100gm provides 325 IU, Eel - 100gm provides 200 IU, Salmon - 100gm provides 345 IU, Sardines - 50 gm. provides 250 IU, Beef liver - 100gm provides 15 IU, Whole eggs - provide 20 IU, fish oil - 15ml provides 1360 IU.

7.1.1 Vitamin D Metabolism

Vitamin D has been identified as a prohormone synthesized in the skin and regulates calcium metabolism after undergoing metabolic conversion to its hormonally active form, 1,25 dihydroxyvitamin D \([1,25(OH)_{2} D]\) in liver and kidney. In skeletal muscles the action of vitamin D is mediated by binding to its receptor. When 1,25(OH)_{2} D interacts with the vitamin D receptor (VDR) it induces heterodimerization of VDR with the retinoid X receptor (RXR). This heterodimer then binds to the Vitamin D response elements in DNA and recruits various coactivators. This leads to the enhanced transcription of genes whose protein products control calcium homeostasis. (J. Barsony, S.J. Marx, 1988) The presence of rapid, transcription-independent events have also been observed in response to physiologic levels of vitamin D and the existence of 1,25(OH)_{2}D-inducible signal transduction pathways are located within
various tissues including adipocytes. \textit{(J.C. Fleet, 2004)} 1,25(OH)_{2}D rapidly (within seconds and minutes) stimulates events, normally associated with the activation of membrane receptors for growth factors and peptide hormones. \textit{(H.L. Gulseth et al, 2010)} These include:

- Phospholipase C (PLC) and Phospholipase D activation.
- Phosphoinositide turnover leading to the generation of the second messengers, inositol 1, 3, 4-triphosphate (IP3) and 1, 2-diacylglycerol (DAG).
- Increase in intracellular calcium by increasing calcium uptake and the release of intracellular calcium stores.
- Adenylate cyclase activation to increase cAMP levels and stimulation of protein kinase A (PKA) activity.
- Calcium-dependent protein kinase C (PKC) activation and cellular redistribution.

7.1.2 Probable Mechanisms of Action of Vitamin D on Glucose Metabolism

Impaired pancreatic $\beta$-cell function, insulin resistance and systemic inflammation are the major causes of the pathogenesis of NIDDM, and vitamin D has been shown to have influence on them as summarized below:

7.1.2.1 Vitamin D and Pancreatic Beta cell function/ Insulin Secretion

Various studies have shown the presence of specific vitamin D receptors (VDRs) on pancreatic $\beta$-cells, the expression of 1-$\alpha$-hydroxylase enzyme in pancreatic $\beta$-cells \textit{(R. Bland et al, 2004)} which catalyzes the conversion of 25(OH)D to 1,25-dihydroxyvitamin D(1,25(OH)_{2}D_{3}), \textit{(B. Maestro et al, 2003)} and the presence of a vitamin D response element in the human insulin gene promoter. All these findings indicate the influence of Vitamin D on glucose homeostasis. The mechanism by which 1, 25(OH)_{2}D_{3} might act on insulin secretion is suggested by the significant rise in cytosolic Ca^{2+} levels. This is observed following 1,25(OH)_{2}D_{3}-stimulated secretion of insulin by islet cells. Pancreatic $\beta$-cells depend on an acute intracellular calcium
increase for insulin secretion (Enju Liu et al, 2009), which may also be attenuated with elevated cytosolic calcium. (C. B. Wollheim and G. W. Sharp, 1981) The controversy remains as to whether an influx of external Ca\(^{2+}\) via voltage-dependent Ca\(^{2+}\) channels is solely responsible for this rise or whether the mobilization of Ca\(^{2+}\) occurs from intracellular organelles via the activation of release of potentiating systems via protein kinase C and protein kinase A pathways.

![Diagram of vitamin D's mechanism of action in increasing insulin sensitivity in peripheral tissues of Type 2 diabetic individual](adapted from springer images).

**Fig 1.4 Mechanism of action of vitamin D in increasing insulin sensitivity in peripheral tissues of Type 2 diabetic individual (adapted from springer images).**

7.1.2.2 Vitamin D and Insulin sensitivity

VDR is also expressed by human skeletal muscle and adipose tissue, (J. C. Fleet, 2004; H. L. Gulseth, et al 2010) the main determinants of peripheral insulin sensitivity. These tissues were, shown to express the 1α-hydroxylase gene in male Wistar rats. (A.L. Sutton, 2003) Notably, skeletal muscle expression of VDR declines with age, and so does the insulin sensitivity. (C. Cade, 1983) Vitamin D deficiency may influence insulin sensitivity via its effects on intracellular calcium. Elevated intracellular calcium impairs post-receptor insulin binding action, such as dephosphorylation of glycogen synthase and of the insulin regulatable glucose
transporter (GLUT-4). (H. A. Bischoff-Ferrari et al, 2004) Vitamin D deficiency results in elevated parathyroid hormone (PTH), which in turn is known to elevate intracellular calcium. Sustained elevations of intracellular calcium may inhibit insulin-target cells from sensing the brisk intracellular calcium fluxes necessary for insulin action, such as glucose transport. (J. E.-B. Reusch, 1991) Therefore vitamin D may improve insulin action by its ability to reduce PTH levels (K. Hida et al, 2005). There is evidence that increased blood PTH is associated with insulin resistance or glucose intolerance. (J.L. Evans et al, 2003; A.C. Maritim et al, 2003) It is well established that there is an increased prevalence of type 2 diabetes mellitus (8 %) and glucose intolerance (40 %) in patients with primary hyperparathyroidism. In addition, fasting PTH levels were shown to be inversely correlated with insulin sensitivity index in fifty-two normotensive, healthy subjects, even after adjustment for potentially confounding factors, supporting the hypothesis that higher fasting serum PTH may mediate a reduction in glucose tolerance. These results support a role of physiological levels of PTH and 1,25 (OH)2D hormones in insulin action. Another possible mechanism is mediated via intracellular calcium, which enhances calmodulin binding to insulin receptor substrate-1 (IRS-1), therefore interfering with insulin-stimulated tyrosine phosphorylation and PI3-kinase activation. (D. S. Worrall and J. M. Olefsky, 2002; M. B. Zemel, 1998) In addition, 1,25(OH)2D3 directly activates transcription of the human insulin receptor gene that activates peroxisome proliferators activator receptor-\(\gamma\) (H. G. Munshi, 1996), that stimulates the expression of insulin receptor, and enhances insulin-mediated glucose transport in vitro.

It has also been found that 1,25 dihydroxyvitamin D3 modulates the rennin–angiotensin system. Although the exact mechanism is not known but studies have reported that vitamin D deficiency leads to an increase in the levels of rennin and angiotensin II synthesis, angiotensin II once synthesized increases the production of reactive oxygen species (ROS) through membrane bound NADPH oxidase and activates small molecular weight G-proteins like Rho A. Rho A inhibits insulin receptor substrate (IRS) phosphorylation thus blocking PKC (Akt) mediated translocation of glucose transporter (GLUT-4) in case of skeletal muscles and adipocytes, and limiting the amount of glucose entering the cell thereby decreasing insulin sensitivity and causing insulin resistance.
The genotypic links of vitamin D to insulin resistance are one the major reasons that help it in maintaining glucose homeostasis. The presence of vitamin D response elements in the promoter region of insulin receptor gene can be one of the reason paving way to improved insulin sensitivity in response to vitamin D supplemenation. Therefore, increase in vitamin D concentration in circulation can directly increase the concentration of insulin receptors on the surface of target tissues. In addition to this, it has been found that the individuals having Bsml (VDR/BB) genotypes have higher levels of fasting glucose as compared to those having genotype (Bb), though some studies have found this association to be conflicting. This finding supports the very idea that not only vitamin D as a molecule but also its receptors and response elements are involved in its insulin sensitizing effects.

7.1.2.3 Vitamin D and Systemic Inflammation

Inflammatory factors have often been associated with insulin resistance and β-cell failure, both of which are the key features of NIDDM. An increase in acute-phase proteins, cytokines and mediators associated with endothelial dysfunction has been reported in NIDDM. In NIDDM, abnormalities in many systemic inflammatory markers have been found, such as tumour necrosis factor (TNF)-α and TNF-β, interleukin-6 (IL-6) and its receptor, C-reactive protein and plasminogen activator inhibitor-1. (B.S. Chertow et al, 1983) Some of these immune mediators, such as TNF-α and IL-6 may directly interfere with insulin signaling, leading to insulin resistance by several mechanisms. Interestingly, vitamin D has been reported to down-regulate the production of several cytokines: IL-2, IL-6 and IL-12, interferon γ, TNF-α and TNF-β. Since a variety of studies have reported that these inflammatory changes may occur many years before diabetes onset, therefore they are regarded as contributors rather than a consequence of the disease.

The reported existence of VDRs in activated T lymphocytes, macrophages and thymus tissue supports the idea that vitamin D may also function as an immune-modulator. Some of the non-classical actions of vitamin D therefore point to the role of this molecule in the pathogenesis of NIDDM.
7.2 Vitamin C

Ascorbic acid is a weak sugar acid structurally related to glucose and is synthesized de novo from glucose in most adult mammals, however some primates and humans cannot synthesize it due to the absence of enzyme L-glucono-δ-lactone oxidase (GULO), which is required in the last step of vitamin C synthesis and thus depend on diet for proper intake of this vitamin. (G. Pittas et al, 2008) The normal levels of vitamin C found in the body of healthy individuals varies between 900-1500 mg. Foods rich in vitamin C include strawberries, papayas, kiwi, bell peppers, guava, melons, dark leafy vegetables which may contain up to 130 mg vitamin C in a single serving, broccoli, cauliflower, tomatoes, 100 gm. of which contain over 100 mg of vitamin C and some herbs like thyme contain 160 mg of vitamin C in one cup.

Vitamin C has many physiological functions including the biosynthesis of collagen, carnitine and neurotransmitters like dopamine. As an antioxidant, it scavenges free radicals and acts as a reducing agent donating electrons and preventing oxidation to keep iron and copper atoms in their reduced states. Apart from these functions vitamin C also acts as a cofactor for eight different enzymes (R. Jenness, 1980): Three of these enzymes participate in collagen hydroxylation (M. Levine, 2000), adding hydroxyl groups to the amino acids proline or lysine in the collagen molecule via prolyl hydroxylase and lysyl hydroxylase. Hydroxylation allows the collagen molecule to assume its triple helix structure, and thus vitamin C is essential to the development and maintenance of scar tissue, blood vessels, and cartilage.

The other two enzymes are necessary for synthesis of carnitine (D.J. Prockop and K.I. Kivirikko, 1995) which is essential for the transport of fatty acids into mitochondria for β-oxidation. The remaining three enzymes have some functions in common in addition to their individual specific actions like dopamine beta hydroxylase participates in the biosynthesis of norepinephrine from dopamine (C.J. Kivirikko, 1991), another enzyme adds amide groups to peptide hormones, greatly increasing their stability (M. Levine et al, 1992) and the last one modulates tyrosine metabolism.
7.2.1 Vitamin C metabolism

Ascorbic acid (AA) after ingestion is absorbed by the gastrointestinal tract. After coming in contact with the oxidants in lumen of gastrointestinal tract, some of the ascorbic acid is converted into its oxidized form dehydroascorbic acid (DHA). (B.A. Eipper et al, 1993) Both AA and DHA are then transported across the cell membrane of enterocytes via specific transporters. The Na⁺- ascorbate cotransporter takes up the AA with high affinity while DHA is transported by facilitated diffusion in a Na⁺ independent low affinity process mediated by glucose transporter family (GLUT). (S.A. Kyrtopoulous, et al, 1991) GLUT 1 and GLUT 3 are the major transporters of DHA located predominantly in osteoblasts, (C. Malo et al, 2000) muscles (S. Qutob et al, 1998) and retinal cells (J. Korcok et al, 2003) however GLUT 4, found in skeletal muscles and adipocytes is also involved in DHA uptake but at a lower level. (K. Hosoya et al, 2004) Once inside enterocyte, DHA is converted back to AA by DHA reductases (S.C. Rumsey et al, 2000), thus maintaining the gradient for continuous uptake DHA, lowering the chances of its escape in the blood stream. Since DHA shares the transporters with glucose, its uptake is competitively inhibited by this monosaccharide. (G.D. Buffinton and W.F. Doe, 1995) Changes in the circulating levels of glucose inhibit the uptake of DHA inside the cell and thus attenuate the bioavailability of vitamin C.

Antioxidant role of Vitamin C

There are two major properties of vitamin C that makes it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate (282 mV) and ascorbyl radical (217 mV), which is derived from the ene-diol functional group of the molecule (Halliwell, 1996). These low reduction potentials enable ascorbate and ascorbyl radical to react with and reduce almost all physiologically relevant radicals and oxidants. The second major property that makes vitamin C an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species. The ascorbyl radical readily dismutates to form ascorbate and dihydroascorbic acid or is reduced back to ascorbate by an NADH-dependent semidehydroascorbate reductase (Wells and Jung, 1997). In this conversion, DHA takes up electrons from the mitochondrial electron transport
chain specifically from complex III thereby decreasing the reduced state of electron transport chain and the subsequent electron leakage finally leading to decreased reactive oxygen species (ROS) formation. (J. Korcok et al, 2003) Another pathway involved in the inter-conversion of DHA to AA is mediated by reduced glutathione (GSH). In this process GSH is converted to its oxidized form (GSSG), which at the expense of NADPH can again be brought back to its reduced form. (X.Li et al, 2002) Thus, DHA conversion to AA indirectly can stimulate pentose phosphate pathway to produce more cellular reductants, decreasing the chances of oxidative stress. Once generated AA can scavenge the ROS directly at the site of generation. Therefore vitamin C shows anti-oxidant effect both during and after conversion.

Fig 1.5 Vitamin C uptake and recycling in mitochondria. Vitamin C in its oxidized form dehydroascorbic acid (DHA) is transported into mitochondria of the isolated kidney cells via GLUT-1. The transported DHA is reduced in the mitochondria. The respiratory chain can donate electrons for the reduction and the site of the reduction is complex III (Li et al., 2002). DHA is also reduced back to ascorbate by DHA reductase and glutathione. The oxidized glutathione is reduced back at the expense of NADPH (Li et al., 2001). DHA reduction can also be mediated by lipoic acid containing complexes (Xu and Wells, 1996). Ascorbic acid (AA) can leave the mitochondria, mediated by a presently unknown transporter. AA quenches ROS protecting the mitochondrial genome and preventing mitochondrial membrane depolarization. (Adapted from J Mandl et al/ British Journal Of Pharmacology; 2009).
7.3 *Vitamin C and Diabetes*

The excess amount of glucose in circulation (hyperglycemia) as in case of diabetes competitively inhibits the uptake of DHA via GLUT transporters inside the cells due to the structural similarity between glucose and vitamin C. This creates an imbalance in the levels of vitamin C which can also become detrimental for the cell. Administration of DHA has been found to protect the neural cells from ischemic stroke by increasing antioxidant levels through GLUT mediated vitamin C accumulation. This may also protect against ROS generated from mitochondrial respiration as mitochondria is the major site for generation of ROS. *(X. Li et al, 2001)*

In normal tissues, insulin increases the facilitated transport of DHA inside the cell but in case of diabetes *(S. Qutob et al, 1998)*, when the cells become insulin resistant, the cellular DHA uptake is impaired leading to decreased regeneration of AA and reduced antioxidant levels thereby increasing the susceptibility of ROS mediated damage of biomolecules. The decrease in cellular reductants provides an ideal niche for reactive oxygen species to oxidize proteins, lipids and DNA, causing diabetes related complications like cardiovascular diseases (CVD) and atherosclerosis.

![Diagram](Image)

*Fig 1.6 In type 2 diabetes, insulin resistance and hyperglycemia together blocks the transport of DHA inside the cell and thus its conversion to ascorbic acid thereby decreasing cellular reductants and increasing oxidative stress (adapted from J.X Wilson/FEBS Letters; 2002).*
7.4 Vitamin A

Vitamin A is a name given to fat soluble, related compounds including retinol, retinaldehyde, retinoic acid etc. Both mammals and other animals depend upon their diet for the intake of this vitamin due to their inability to synthesize it. The two major forms of vitamin A present in foods are:

- Retinol, a yellow colored fat soluble substance is obtained from foods of animal origin like fish, meat (especially liver) and dairy products. Since retinol (a pure alcohol form) is an unstable form of vitamin A, it is generally found in the form of retinyl esters mostly retinyl palmitate or retinyl acetate.

- Carotenes are the form of vitamin A obtained from foods of plant origin like carrots. However, carotenes lacking beta-ionone rings cannot be converted into vitamin A by herbivorous and omnivorous animals, while humans possess the enzyme beta carotene 15-15' monoxygenase and thus convert alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll beta-cryptoxanthin (all of which contain beta-ionone rings) to vitamin A.

7.4.1 Vitamin A biosynthesis

The first committed step in plant carotenoid synthesis is the condensation of two molecules of GGDP (geranylgeranyl diphosphate) to produce phytoene (Figure 1.7) by the enzyme phytoene synthase (PSY). (P. Beyer, 1989) Phytoene is produced as a 15-cis isomer, which is subsequently converted to all-trans isomer derivatives. Two plant desaturases, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), catalyze similar dehydrogenation reactions by introducing four double bonds to form lycopene. (P. Carol et al, 1999) Desaturation requires a plastid terminal oxidase and plastoquinone in photosynthetic tissues. (E. Collakova, et al, 2003) Lycopene once formed is then acted upon by lycopene cyclase generating α-carotene and β-carotene. Both these carotenes act like vitamin A precursors or provitamin A and are a substrate for enzyme beta carotene 15-15' monoxygenase, found in animals and humans which convert them to vitamin A (Retinol). (Dean Della Penna and Barry J. Pogson, 2006)
Vitamin A metabolism begins with the physical release of retinol, retinyl esters or carotenoids from the diet. All these three forms of vitamin A undergo different stages before they are released in circulation. Retinol after entering the lumen undergoes emulsification by fatty acids and bile acids in order to enter enterocytes, however retinyl esters in the lumen are acted upon by two enzymes; pancreatic triglyceride lipase (PTL) and pancreatic lipase related protein 2 (PLRP2), which covert them into retinol which can enter directly into the enterocytes whereas the conversion of carotenoids to retinol taken place in the enterocytes itself. (H. Earl Harrison, 2005) Carotenoids in the enterocytes undergo both symmetrical and asymmetrical cleavage by \( \beta \) carotene 15-15’monooxygenase and \( \beta \) carotene 9’-10’monooxygenase respectively yielding retinol and \( \beta \) apo-carotenoids. (JA Olson, 1989) Finally, retinol in the
enterocytes undergoes esterification by the action of two enzymes; 1. Lecithin: retinol acyltransferase (LRAT) which performs the trans-esterification of retinol by employing the fatty acyl group present at the A1 position of a membrane phosphotidyl choline molecule for its packaging into chylomicrons. 2. Acyl-CoA: retinol transferase (ARAT) which carries out the fatty acyl CoA dependent esterification of retinol. \( \text{(R.K. Randolph, } \text{et al, 1991)} \) After being esterified, retinol is released in the circulation in the form of retinol bound chylomicrons, which are taken up by liver for storage and release depending upon the level of vitamin A in circulation. \( \text{(S. Vogel et al, 1999)} \)

Fig 1.8 The metabolic pathway of Vitamin A (adapted from Theo J. C. van Berkel/journal of lipid research; 2009).

7.6 Vitamin A and Diabetes

Vitamin A is required for normal growth, vision, and resistance to infection. According to studies, vitamin A also plays a role of cardinal importance in processes like(i) stimulating glucose transporter expression in L6 muscle cells, \( \text{(R. Blomhoff and H.K. Blomhoff, 2006)} \) (ii) restoring insulin secretion in vitamin A deficient mice, \( \text{(M.W. Sleeman et al, 1995)} \) (ii) inducing both first and second phase insulin secretory responses to glucose in explants of human fetal pancreas, \( \text{(B.S. Chertow et al, 1987)} \) (iv) increasing insulin production in RINm5F cells and human islets and last but not the least \( \text{(B.E. Tuch and K.J. Osgerby, 1990)} \) (v) stimulating glucokinase gene expression and enzyme activity in pancreatic islets and improving glucose sensing system. Therefore, its deficiency can affect the physiological functions required for
proper functioning of the body. Several studies have found a deficiency in the status of vitamin A (especially in levels of all-trans retinoic acid and 9 cis-retinoic acid) in type 2 diabetic patients, thereby increasing their oxidative stress and also enhancing their probability of developing other complications related to insulin secretion and sensitivity.

After having an understanding of the mechanisms and risk factors underlying the incidence, development and progression of type 2 diabetes mellitus and possible related complications, extensive research should be extended to fight this deadly disease. From a pathophysiological standpoint, the two major players of T2DM are hyperglycemia and oxidative stress. Hyperglycemia serves as a root cause of all the underlying complications by mediating its effects in more than one ways. (P. Anabela Rolo, M. Carlos Palmeira, 2006; L. George King, R. Mary Loeken, 2004) All the pathways however, converge to ultimately give rise to the major player: oxidative stress in the pathogenesis of T2DM. Once generated, the free radicals cause pancreatic β cell damage, thus increasing hyperglycemia, mitochondrial dysfunction, lipid peroxidation, glucose oxidation, DNA damage and lastly, manifesting changes in the histology of diabetic tissues. (A. Ceriello, 2003) In order to stop the progression of the disease or to slow down the occurrence of diabetic complications, these reactive oxygen species (ROS) should either be quenched or their production must be downregulated. (J.L. Evans et al, 2003; A.C. Maritim et al, 2003) However, it will be of double benefit if T2DM complications like hyperglycemia as well as oxidative stress, are targeted simultaneously. With this approach we studied the effect of several vitamins (Vitamin D, Vitamin C and vitamin A) alone and in combination of each other in type 2 diabetic swiss albino mice. The sole purpose of taking different vitamins was to see their individual effect since all three vitamins have different modes, sites and targets of action.

Therefore, in this study conducted, we not only observed the parameters related to glucose metabolism but also those involved in oxidative stress, since both are interrelated and equally important in order to come up with a vitamin or a combination with highest anti-diabetic potential.