Chapter 1
Introduction
1.1. Diabetes mellitus

Glucose is a simple sugar and an important metabolic intermediate that serves as the fuel of the body. Most of the food we are consuming is converted into glucose in the stomach through a series of enzymatic digestion and it enters into blood through specific glucose transporters. The glucose receptor cells in the pancreas monitor the blood glucose concentration and pancreas plays an important role in the maintenance of blood glucose homeostasis by secreting hormones like insulin and glucagon. Among these, insulin is the most important regulatory hormone that mediates the transport of glucose into the peripheral tissues such as adipocyte and muscle, where glucose is further metabolized to release energy or stored as glycogen. In case if glucose is not entered in to the glucose metabolizing cells, blood glucose level will increase and that eventually leads to severe metabolic disorders such as diabetes mellitus. Diabetes is often characterized by persistent hyperglycemic condition in the body. This increased blood sugar level in diabetes is either because the body does not produce enough amount of insulin or because the sugar disposal system of the body does not respond to insulin as in the normal case. These physiological facts reveal a complex but intensely regulated system in the body for the maintenance of blood glucose homeostasis involving pancreas, adipocyte, muscle and liver and any malfunction of these tissues leads to the development of diabetes mellitus.

1.1.1. Types of diabetes

Diabetes in general, has been classified into three types namely type I, type II, gestational and other specific types of diabetes. Type I diabetes, which accounts for 5-10% of all cases of diabetes, is affected with various microvascular and macrovascular complications that leads to the major morbidity and mortality associated with it [1]. Type 1 diabetes can start at any age and the pathogenecity is strongly associated to the autoimmune problems against the β cells of islets of Langerhans that ultimately result in decreased insulin production [2]. General symptoms of type 1 diabetes include increased thirst and urination, hunger, weight loss and extreme fatigue.
Although type 1 diabetes has an increase in its prevalence, the most common type of diabetes is type 2 diabetes mellitus (T2D) that accounts for more than 90% of all diabetic cases in the world. Statistics of world health organization (WHO) show that India is more pronounced in diabetic epidemic than any other country as India had 32 million people with diabetes in the year 2000 [3]. According to the International Federation of Diabetes (IDF) statistics, the estimated prevalence of diabetes in South-East Asia by 2010 is 58.7 million with an increase to 101 million by 2030. About 85% of the adult population of South East Asia is accounted by India and the prevalence of diabetes in Indian population is estimated to be around 50.8 million [4, 5]. Unlike type 1 diabetes in which insulin production is affected mainly due to the autoimmune destruction of β cells, T2D is characterized by insulin resistance, a phenomenon, where even in the presence of sufficient amount of insulin, it fails to exert its effect on the peripheral tissues like adipocytes and muscle. Not only that, it is observed that the insulin sensitivity will be decreased in peoples who are normoglycemic first degree relatives of patients with T2D and they are known to be at risk of T2D [6,7]. It is well established that insulin resistance is associated with generalized and abdominal obesity [8] along with other factors like accumulation of hepatic fat and intra-myocellular lipids [9]. All these findings point out the association of obesity and lipid accumulation with insulin resistance and T2D. Hence the best way to counteract insulin resistance is to reduce the obesity and fat deposition through exercise and proper diet or the use of chemical agents that can improve the action of insulin in peripheral tissues. Both exercise and insulin sensitizing agents are shown to have regulatory effect on insulin signaling pathway to stimulate glucose uptake in to adipocytes and muscle.

1.1.2. Insulin and insulin mediated glucose disposal

Insulin is a 5808 Da peptide hormone composed of 51 amino acids. The name insulin comes from the Latin word *insula* for "island" and as the name suggests, the hormone is secreted by the β cells of islets of Langerhans in the pancreas whenever there is a rise in blood glucose level. Dietary carbohydrates are converted into glucose in the gut through various enzymatic breakdowns and the glucose enters into the blood through glucose transporter (GLUT) 2 expressed in the brush border membrane of the small intestine. This rise in blood glucose level triggers the secretion of insulin from pancreatic
β cells. Glucose receptor cells in the pancreas detect the rise in blood glucose level and allow the glucose to enter into the β cells through GLUT2. The transported glucose is metabolized through a glycolytic pathway and results in the generation of ATP. This causes a change in the ATP:ADP ratio leading to the closure of K⁺ channel and depolarization of the membrane followed by the activation of voltage gated calcium channel to open up, allowing the influx of calcium ions into the β cells. This Ca²⁺ influx, through various signal transduction pathways, stimulates the secretion of insulin and it is carried to the insulin sensitive tissues where they stimulate the uptake of excess glucose present in the blood stream. Fig: 1.1 gives a diagrammatic representation of insulin secretion by pancreatic β cells.

![Diagram of Insulin Secretion](http://www.betacell.org)

**Fig.1.1: Insulin secretion** – Entry of glucose into the β cells through GLUT2 causes a rise in the ATP:ADP ratio. This depolarizes the membrane causing the Ca²⁺ channel to open up. Rise in calcium level leads to the exocytotic release of insulin from their storage granule. *Figure courtesy: http://www.betacell.org.*

1.1.3. **Insulin signaling and insulin resistance**

Insulin regulates various functions in the body. The most important action of insulin is to increase the storage and utilization of energy mainly by regulating the
transport of glucose into cells [10]. Insulin increases the glucose uptake in various tissues by increasing the membrane translocation of glucose transporter 4 (GLUT4), a facilitative glucose transporter isoform [11]. Binding of insulin to the insulin receptor (IR) induces transphosphorylation and autophosphorylation of β subunit on specific tyrosine residues, leading to the phosphorylation of tyrosine residues on various intracellular substrates including insulin receptor substrate family (IRS1 to 4), Cas-Br-M (murine) ecotropic retroviral transforming sequence homologue (Cbl) and Adaptor Protein containing SH2 & PH2 domains (APS) [12,13]. At this point the insulin stimulated glucose uptake follows a phosphatidylinositol-3-kinase (PI3K) dependent and independent pathway. The classical insulin signaling follows the PI3K dependent pathway in which phosphorylation of IRS proteins leads to the activation of PI3K, followed by the phosphorylation and activation of several downstream kinases like protein kinase B (PKB/Akt) and atypical protein kinase C (aPKCζ/λ) [14]. Eventhough the importance of PI3K is evident, it is suggested that the activation of PI3K is not sufficient for a complete stimulation of glucose uptake [15, 16]. Several studies have shown that IR residing in the lipid raft microdomains follow separate signaling pathway through the stimulation of tyrosine phosphorylation on Cbl proteins [17, 18]. This is considered as a second insulin receptor signaling pathway that functions in concert with IRS-PI3K pathway. Later studies revealed the importance of Cbl adaptor proteins APS and Cbl associated protein (CAP) and their role in insulin induced Cbl phosphorylation leading to increased glucose uptake through increased GLUT4 translocation [19, 20].

Insulin regulates glucose homeostasis not only by increasing the uptake of glucose into muscles and adipocytes, but also by reducing hepatic glucose release by reducing gluconeogenesis and glycogenolysis [21]. In addition to these, insulin affects the lipid metabolism by increasing lipid synthesis in fat and liver cells and by reducing the fatty acid release from fat and muscle cells [22]. Regulation of these various biological processes by insulin is important for the normal functioning of the body and any defect in these processes leads to insulin resistance, a condition where insulin fails to give normal response at its physiological concentration. In order to overcome this, body secretes more amount of insulin leading to a hyperinsulinemic condition, which is characteristic of insulin resistance. Fig.1.2 gives a schematic representation of insulin response in normal and insulin resistant stages. Insulin resistance in peripheral tissues like muscle and
adipocytes will affect the insulin mediated GLUT4 translocation and glucose uptake leading to an increase in blood glucose concentration and finally leads to T2D [23].

**Fig.1.2: Insulin response** - A schematic representation of insulin response in peripheral tissues in normal and insulin resistant stage.

### 1.1.4. Insulin resistance in adipocytes and muscles

Adipocytes and muscles are the major sites of insulin mediated glucose disposal and these tissues known to express high levels of insulin sensitive glucose transporter isoform called GLUT4. Following a high carbohydrate diet, the insulin secreted by pancreas will activate the insulin receptors on adipose and muscle cells resulting in increased membrane translocation of GLUT4 proteins residing in the intracellular compartments [24, 25]. In insulin resistant conditions, such as in T2D and obesity, this insulin stimulated GLUT4 translocation and glucose uptake is strikingly impaired in adipocytes and muscles [23]. Although the exact mechanism that leads to insulin resistance in peripheral tissues is not clearly understood, the defects in glucose metabolism in insulin resistant tissues are associated with various cellular functions like insulin signaling, glucose transport and glucose metabolism. Under insulin resistance state, it has been shown that tyrosin phosphorylation of IR and IRS is reduced thereby
affecting GLUT4 translocation and glucose uptake [26]. In addition to glucose transport, glucose metabolism is also affected in insulin resistant cells by decreased glucose phosphorylation and impaired glycogen synthesis [27, 28].

Glucose and free fatty acid (FFA) are the major sources of energy for skeletal muscle. During fasting stage, muscle glucose uptake is low and the plasma FFA concentration is elevated due to the lipolysis in adipocytes. This FFA serves as the principal energy source for muscle energy production during fasting [29]. Following glucose ingestion the increased plasma glucose concentration results in insulin mediated suppression of lipolysis and subsequent decrease in the rate of lipid oxidation [30]. Insulin increases the muscle glucose uptake leading to an increase in muscle glucose oxidation. This metabolic flexibility is affected in insulin resistant state leading to a significant reduction in insulin mediated glucose uptake in skeletal muscle [31]. In skeletal muscle, both decreased mitochondrial fat oxidation and increased FFA influx take place in the insulin resistant state. These fatty acid metabolites impair IRS1 phosphorylation by IR leading to a defective insulin signaling. FFA can disrupt the downstream effectors of insulin signaling by inhibiting insulin mediated activation of Akt/PKB, which was evidenced by the inhibition of insulin mediated Akt activation when saturated fat is administered in cultured muscle cells [32]. The major biological and biochemical effect of insulin signaling in muscles and adipocytes is the increased translocation of GLUT4 and glucose uptake and insulin resistance caused by various factors substantially reduced these processes. This will lead to a persistent increase in blood glucose concentration and ultimately to diabetes. Hence glucose transporters and regulation of glucose transporter mediated glucose uptake in peripheral tissues become one of the key targets to counteract the effects insulin resistance and associated metabolic abnormalities.

1.2. GLUCOSE TRANSPORTERS

The high polar nature of glucose makes it impermeable to transport across the membrane and therefore specific carrier molecules on the cell membrane are required for this purpose. Movement of glucose across the membrane takes place in an energy dependent (active) as well as energy independent (passive) manner. Attempts to
understand the mechanism and nature of glucose transport had been started early in 1948 as LeFevre was the first to postulate that specific components within the plasma membrane are required for the transfer of glucose across the membrane [33]. In the late 1970s and in 1980s major works have been carried out and glucose transport across the membrane was demonstrated to be mediated through transmembrane proteins, and was partially purified and functionally reconstituted [34, 35]. Later in 1985 cDNA encoding red cell glucose transporter was cloned and following that, 13 related members of glucose transporter proteins have been identified in human being [36, 37]. The glucose transporters are 12 transmembrane proteins belong to the Major Facilitator Superfamily (MFS) of membrane transporters. The members of Glut family have been classified into different classes and isoforms based on their sequence similarity and physiological role [38, 39].

1.2.1. Glucose transporter isoforms.

Out of the 14 isoforms of glucose transporters identified so far, class I glucose transporters include GLUT1, GLUT2, GLUT3, GLUT4 and GLUT14 [40]. In this GLUT1 is a ubiquitously expressed protein and is the first one to be sequenced and purified and has been subjected to intense biological and biochemical investigations [41, 42, 43]. GLUT2 expressed in high amount in various cells like pancreatic β cells, basolateral membranes of intestinal and kidney cells. As evidenced from its distribution pattern, GLUT2 plays critical role in the absorption of glucose from the intestine and also plays critical role in triggering insulin secretion in pancreatic β cells. Absence or improper regulation of GLUT2 results in increased blood glucose concentration mainly because of the diminished secretion of insulin [44, 45, 46]. GLUT3 and GLUT14 are considered as much similar in its biochemical and structural aspects and are expressed in neuronal cells and testis respectively. GLUT3 has high affinity to glucose and highest turn over number ensuring efficient glucose uptake by neurons [47, 48]. Among the various GLUTs in class I, GLUT4 is the major insulin sensitive isoform predominantly expressed in adipocytes and muscle cells [49].

The other GLUT isoforms belonging to class II and III include various fructose transporters and glucose transporters expressed in various tissues like kidney, liver,
placenta, cardiac and skeletal muscle, leukocyte, blasocyte, brain, glial cells and some neurons [40].

1.2.2. GLUT4 – structural and biochemical aspects

GLUT4 is a 54kDa protein consisting of 509 amino acids arranged as 12 transmembrane domains. The protein was discovered as distinct insulin sensitive transporter isoform in the late 1980s [49] and several studies have been carried out in that decade to understand more on insulin mediated GLUT4 translocation in muscles and adipocytes [50, 51, 52]. GLUT4, after its biogenesis, is targeted to specific membrane compartments that are insulin sensitive and these compartments are the primary site of insulin action [53]. Molecular level understandings show that in insulin resistance, recruitment of GLUT4 to the plasma membrane is affected than the normal GLUT4 protein expression [54, 55]. These findings emphasize the importance of understanding various structural, functional and molecular aspects of GLUT4 and insulin regulated GLUT4 translocation and glucose uptake so as to understand important key nodes associated with insulin resistance.

![GLUT4 Structure](image)

**Fig.1.3: GLUT4 structure** - Schematic representation of the topological structure of GLUT4. The 12 transmembrane segments are shown. The important motifs in GLUT4 are marked.

Eventhough there is no experimentally determined structure for GLUT4s, the hydropathy analysis suggests that all glucose transporters including GLUT4, share a common topology consisting of 12 transmembrane domain segments [36]. Fig: 1.3 gives
a schematic representation of the topological structure of GLUT specifically showing important motifs present in GLUT4. There are several specific signature sequences present in GLUT4 that are important for the regulation of GLUT4 trafficking and glucose transport. Unlike GLUT1, which localizes predominantly in the plasma membrane, GLUT4 resides in the intracellular vesicles in the basal state. This suggests the presence of intrinsic targeting domains in GLUT4 that directs its localization to the insulin responsive compartments [56].

Intra cellular amino and carboxy termini of GLUT4 have gained lot of attention in this regard. Though some early studies showed that these termini does not contribute to the intra cellular trafficking of GLUT4, several findings came in the later periods have shown that these termini have residues important for the regulation of intracellular GLUT4 trafficking [57]. The FQQI motif in the cytosolic N-terminal domain was shown to be important for the GLUT4 internalization and intracellular sequestration. Detailed studies on FQQI motif later showed that this motif plays significant role in the endocytosis of GLUT4 from the plasma membrane rather than its intracellular sequestration [58, 59, 60, 61]. In addition to this, the dileucine (LL) motif in the C terminus also found to have important role in the endocytosis of GLUT4 from the plasma membrane and in the exit of GLUT4 from TGN [62]. Similar to FQQI motif, mutation of LL to AA was found to significantly inhibit internalization of GLUT4 resulting in the accumulation of mutant GLUT4 protein on the cell surface [63, 64]. Apart from this, a very important and highly conserved motif seen in GLUT4 is QLS motif in the transmembrane helix 7, which is known to interact with glucose and plays an important role in the regulation of glucose transport [65, 66].

As an attempt understand more on the structural details of GLUT4, our laboratory has generated a homology model of GLUT4 based on the experimental data available on GLUT1 and the crystal structure available on glycerol-3-phosphate transporter [67]. The model identified regions in GLUT4 that form a channel for the transport of glucose along the substrate interacting residue. The model was further validated with known substrate of GLUT4 like D-glucose and inhibitors such as cytochalsin B and genistein. This well validated GLUT4 homology model was further used for the molecular dynamic simulation studies to gain an insight into the intrinsic
dynamic behavior, substrate-induced conformational changes and the role of ATP in the regulation of GLUT4-mediated glucose transport. It is observed that in the apo form, the transporter attains a conformation open to the extracellular region and this conformation was found to facilitate the exofacial binding of the substrate and its translocation to the cytoplasm. This study further provided an explanation for the role of ATP in GLUT4 mediated glucose transport [68].

1.2.3. Genetics of GLUT4

The glut4 gene is a member of the solute carrier family 2 which is present at chromosome number 17. Studies from Bell et al reported a region of 8,000 bp coding 11 exons as the glut4 gene [69]. Mutations in this gene have found to be associated with non insulin dependent diabetes mellitus (NIDDM) and various studies have reported the molecular and biochemical implications of these mutations [70, 71]. A valine to isoleucine mutation in GLUT4 was found to be associated with T2D [72]. Similarly, a recent study from our laboratory has also revealed significant association of a common haplotype in GLUT4 genes with type 2 diabetes [73].

1.2.4. Cell biology of GLUT4

Various studies on genetically engineered mouse models, where expression of GLUT4 is either enhanced or ablated in adipocytes or muscles, have clearly demonstrated the pivotal role for GLUT4 on whole body glucose homeostasis. Overexpression of human glut4 gene in diabetic (db/db) mice showed betterment in insulin resistance and diabetes. However, genetic ablation of GLUT4 and GLUT4 null mice did not show severe insulin resistance and diabetes [74, 75]. But at the same time, heterozygous mice (GLUT4+/−) with decreased GLUT4 expression in adipocytes and muscles have shown to be insulin resistant and hyperinsulinemic and overexpression of GLUT4 protein in such animals normalizes insulin sensitivity and glucose tolerance [76, 77]. As muscles and adipocytes are the major insulin sensitive tissues, a conditional depletion of GLUT4 in any of these tissues causes insulin resistance leading to diabetes. Studies have shown a possible crosstalk between adipocyte and muscles in insulin resistance, as conditional depletion of GLUT4 in muscle cells cause decreased insulin sensitivity in adipose tissue.
and *vice-versa*. Genetic ablation of GLUT4 gene specifically in muscles resulted in insulin resistance and glucose intolerance in mice and disruption of GLUT4 in adipocyte resulted in impaired glucose uptake along with insulin resistance in muscle and liver [78, 79]. These data reinforce the dominant role of GLUT4 as a regulator of whole body glucose homeostasis, and acute or long term changes in the expression of GLUT4 on the surface of adipocyte and muscle cell membrane can lead to systemic changes in the glucose homeostasis.

1.2.5. Membrane translocation of GLUT4

In basal state, GLUT4 resides within intracellular vesicles and undergoes regulated exocytosis in response to various stimuli [80]. The most important factor that stimulates membrane translocation of GLUT4 is the action of insulin. It has also been shown that exocytosis of GLUT4 can be induced by various stimuli such as exercise or muscle contraction. All these responses results in the increased expression of GLUT4 on the plasma membrane and that lead to increased glucose uptake into the cells (Fig.1.4). Among these, the most important pathway that regulates GLUT4 translocation is mediated through insulin signaling.

![Fig.1.4: GLUT4 translocaiton signals](image)

- A schematic representation of various signaling pathways that stimulate GLUT4 translocation in adipocytes and muscles
1.2.5.1. Insulin mediated GLUT4 translocation

Insulin has two major means to increase the expression of GLUT4 on the plasma membrane; one is to increase the exocytosis of GLUT4 storage vesicle (GSV) and the other is to decrease the rate of endocytosis of membrane GLUT4 [81, 82]. Insulin mediated translocation of GLUT4 starts with the binding of insulin to IR and the activation of classical PI3K pathway through the phosphorylation of IRS proteins. A transient increase in the plasma membrane phosphatidylinositol-3,4,5-triphosphate (PIP3) as a result of PI3K mediated conversion of PIP2 to PIP3 leads to the recruitment of PH domain containing proteins like PKB and phosphoinositide dependent kinase (PDK) 1 to the plasma membrane [83, 84]. The recruited PDK1 phosphorylates and activate several downstream kinases including PKB and aPKC [85]. In addition to this, mammalian target of rapamycin (mTOR) complexed to rictor, which is identified as PDK2, can also phosphorylate Akt on Ser^{473} [86]. Several studies have shown the important role of PKB/Akt in insulin mediated GLUT4 translocation. Overexpression of membrane bound Akt increases GLUT4 translocation and glucose transport in adipocytes whereas, insulin mediated GLUT4 translocation can be inhibited by expressing a dominant negative mutant of Akt [87, 88, 89, 90, 91, 92]. Similar to Akt, its downstream effector AS160 (Akt substrates of 160 kDa) has also gained much attention and identified as an important target in the insulin signaling pathway. Though there are several putative substrates for Akt that regulate the glucose transport, AS160, a Rab-GAP (GTPase activating protein), is the most extensively studied molecule [93]. The significance of insulin mediated AS160 phosphorylation has been shown by overexpression studies. AS160 mutant that can not undergo phosphorylation blocks the insulin mediated GLUT4 translocation [94]. Also, AS160 phosphorylation was found to be reduced in patients with type 2 diabetes [95, 96, 97].

Eventhough PI3K acts as an important regulatory enzyme in the insulin mediated GLUT4 translocation, as discussed in the paragraph 1.1.3., activation of PI3K alone is not sufficient for bringing the glucose transporter activity in response to insulin. Several studies suggest a second insulin signaling pathway which is wortmannin insensitive that mediate GLUT4 translocation. IR proteins are found to reside in the lipid raft microdomains possibly through their interaction with raft protein caveolin [17]. Binding
of insulin to these receptors stimulate the tyrosine phosphorylation of c-Cbl and Cbl-b through the phosphorylation of adaptor protein APS. APS is phosphorylated on C-terminal tyrosine resulting in the recruitment and phosphorylation of Cbl on tyrosine residues [18, 19]. This activation further forms a complex with the Cbl-associated protein (CAP) that results in the activation of small GTPase TC10 and it is suggested that this CAP-Cbl pathway and the PI3K pathway stimulate independently and converge for the stimulation of GLUT4 translocation [20, 98].

Atypical PKCζ/λ is known to play an important role in both these pathways that stimulate GLUT4 translocation. Insulin stimulates the PDK1 dependent phosphorylation of threonine residue (Thr^{410}) in the activation loop of aPKC. Constitutively active and dominant negative mutants of aPKCζ/λ were found to affect insulin mediated GLUT4 translocation suggesting a significant role for aPKCζ/λ in the insulin mediated GLUT4 translocation [99, 100, 101, 102]. The activation of PKCζ by the formation of polyphosphoinositides demonstrates PKCζ as downstream of PI3K and it is evidenced by the sensitivity of PKCζ activation by pharmacological inhibitors such as wortmannin [103]. In addition to this, aPKCζ/λ also reported to act as a downstream target for TC10 and these studies indicate that aPKCζ/λ may serve as a convergent downstream target for IRS-PI3K and Cbl-TC10 pathway in adipocytes [104, 105].

1.2.5.2. Insulin independent GLUT4 translocation

It is well established that insulin plays a pivotal role in GLUT4 translocation and glucose uptake in adipocytes and muscle. However several studies have shown that GLUT4 translocation can also be induced by various other stimuli through an insulin independent signaling pathway. Hyperosmolarity is one such stimulus that has shown to induce GLUT4 translocation in a PI3K independent but tyrosine kinase dependent manner in peripheral tissues [106, 107]. Similarly, osmotic shock, hypoxia and exercise are the other major known stimuli that stimulate GLUT4 translocation and glucose uptake in muscles and adipocytes in an insulin independent manner [108, 109, 110, 111]. Delineation of the signaling pathway activated by these stimuli identified AMP-activated protein kinase (AMPK) as a key molecule that regulates GLUT4 translocation. Activation of AMPK in these pathways result mainly from increased AMP:ATP ratio that directly
activates various downstream effectors to induce GLUT4 translocation and glucose uptake in adipocytes and muscles [112, 113, 114]. Studies using various animal models have confirmed the existence of AMPK mediated pathway regulating the GLUT4 translocation. Isolated muscles from insulin resistant Zuker rats exhibited normal response to contraction stimulated glucose uptake whereas muscles from normal mice overexpressing kinase dead AMPK has shown reduced sensitivity to contraction mediated glucose uptake but has normal insulin stimulated glucose transport [115, 116, 117]. It is interesting to note that transgenic mice lacking IR or Akt2 were found to have reduced glucose uptake in response to insulin but are normal with contraction stimulated glucose transport [118, 119]. Later studies found that similar to insulin, exercise or contractile activity can also phosphorylate AS160 suggesting a possibility that AS160 can act as a point of convergence in insulin as well as contraction mediated GLUT4 translocation and glucose uptake [120]. In addition to their role in increased GLUT4 translocation, these stimuli such as muscle contraction and depolarization are also responsible for reducing the rate of GLUT4 endocytosis in muscle cells and thereby increasing the total plasma membrane content of GLUT4 [121, 122, 123].

1.2.6. Signaling pathways in GLUT4 translocation and their importance.

As discussed earlier, diabetes is characterized by a persistent increase in blood glucose concentration and insulin resistance is a characteristic feature of type 2 diabetes. Ultimately in diabetes, glucose disposal is reduced mainly due to defects in insulin signaling and associated defects in GLUT4 translocation in peripheral tissues such as muscles and adipocytes. Eventhough insulin independent pathways are also stimulating GLUT4 translocation and glucose uptake these pathways need not be completely independent and parallel. So in order to counteract insulin resistance and type 2 diabetes, it is important to know the various signaling pathways and effectors involved in GLUT4 translocation and glucose uptake, so as to focus these critical nodes as targets for developing new treatment strategies.
1.3. Treatment for Diabetes

Diabetes as a disease has been recorded in historical time and is characterized by various symptoms observed in human being. Symptoms like polyuria and polydypsia were described in the Egyptian Ebers papyri, Greek Epidemics Book III of Hippocrates and in the Chinese Nei Ching [124, 125]. In Indian scenario, early medical practices like Ayurveda give a detailed description on diabetes (referred as Madhumeha) where symptoms like polyuria and polydypsia are specifically described as characteristics of diabetes. Symptoms like glucose urea and acetone smelled breath was used to differentiate inherited and obesity dependent diabetes, as in modern times, type I and type II respectively [126]. Both traditional and modern medical systems suggest the use of various combinations of drugs for the prevention and treatment of diabetes. However, beyond doubts these practices agree the importance of having a balanced life style and physical activity to prevent and cure diabetes. Cellular and molecular level studies on diabetes also points out the importance of physical activity (exercise) for diabetic patients and it is found that such activity can enhance glucose transport in peripheral tissues in an AMPK dependent pathway.

1.3.1. Insulin for diabetes – advantages and disadvantages.

Type 2 diabetes is usually seen in people who are overweight and in particular those who follow a sedentary life. They are generally insulin resistant and most of the time require higher amount of insulin to maintain the blood glucose level. In insulin resistant case, normal level of insulin failed to exert normal response. In the initial stages, pancreatic β cells manage insulin resistance by secreting more amount of insulin to maintain the blood glucose homeostasis. This gradually leads to hyperinsulinemia resulting in the destruction and dysfunctioning of pancreatic β cells, culminating in hyperglycemia and type 2 diabetes. Therapeutic algorithms suggest that insulin resistance has to be treated during the early phases of disease when pancreas maintains its ability to secrete insulin. Insulin analogues and insulin secretagogues are generally effective in the treatment of type 1 diabetes mellitus as it is caused mainly by the decreased production of insulin due to the autoimmune destruction of pancreatic β cells [127]. Insulin secretagogues stimulate pancreas to secrete more insulin by binding to sulfonylurea
receptors namely sulfonylureas. Rapid acting and long acting insulin analogues such as Insulin Lispro, Insulin Aspart, Insulin Glargine and Insulin Detemir are commonly used for the treatment of Type 1 diabetes mellitus [128, 129, 130, 131].

In addition to this, insulin sensitizers are also commonly used that makes peripheral tissues such as muscles, adipocytes and liver more sensitive to insulin action. These are much more important in the treatment of type 2 diabetes as they have the potential to counteract insulin resistant in the peripheral tissues. The common ones in this group are metformin, from the group called the biguanides and thiazolidinediones (TZDs) or glitazones including rosiglitazone and pioglitazone [132, 133]. Among these metformin increases insulin sensitivity in peripheral tissues and TZD decreases insulin resistance in muscles and adipocytes through increasing the production of GLUT4 [134].

However, these agents and treatment modes found to possess various disadvantages also. Physiological problems like weight gain and hypoglycemia are the major issues associated with insulin analogues. Insulin sensitizers and insulin secretagogues are sometimes suspected for their potential carcinogenic nature. Not only that the cost and practical difficulties of administration of these compounds lead the scientific world to give more attention in developing new treatment strategies which are cost effective and with reduced side effects [135].

1.3.2. Herbal medicines and natural products in the treatment of diabetes

The term herbal medicine is generally used to indicate various kinds of alternate medical practices that explore the use of plants and plant based formulations for the treatment of various diseases. India is well-known for the existence and successful exploration of its diverse alternate medical practices like Ayurveda, Siddha and various tribal medicines from different parts of the country. Although plants and plant based formulations used in these alternate medicines are considered as rich source of potential bioactive molecules, most of the time, the active ingredient and the mode of action of these drugs remain mysterious. Understanding more on these herbal medicines with respect to their active ingredients and mode of action leads to the identification of new molecules that can be used for the treatment purposes. In addition to this, understanding
the mode of action of these drugs can shed light to the molecular and biochemical aspects of various diseases, helping in the identification of new targets for therapeutic purposes. Increasing number of research articles on the antidiabetic effect of phytochemicals in the recent years clearly indicate the emerging importance of this area.

As diabetes is a lifestyle related disease, alternate medicines have significant role in the treatment of diabetes. Ayurveda alone gives a detailed study of nearly 150 plants which can be used as anti diabetic and the number will be much more including other alternate medical practices. Several studies have been carried out with antidiabetic plants in *in-vivo* and *in-vitro* systems with various intentions like; 1) to establish the antidiabetic effect of plants reported in traditional medicines, 2) to understand the mode of action of antidiabetic plants, 3) to isolate and characterize the active component from the plant and 4) to elucidate molecular mechanism through which the extract and/or the active compound exert its effect both *in-vivo* and *in-vitro*. Since most of these studies used a crude extract or a purified fraction of the crude extract for analysis, our laboratory had initiated an attempt to isolate active component from the antidiabetic plants in a bioassay directed manner and to characterize the mode of action. As discussed earlier, GLUT4 translocation is a necessary step for the glucose disposal in peripheral tissues and compounds that can increase the exocytosis or decrease the endocytosis of GLUT4 result in increased amount of GLUT4 on the plasma membrane that lead to increased glucose transport. Hence our laboratory used GLUT4 as the target for screening potential antidiabetic compounds from medicinal plants. Although some of the recent research works have reported a few compounds with GLUT4 translocation and glucose uptake activity from medicinal plants, the work reported here was the first attempt where GLUT4 translocation was used as a bioassay for the screening and identification of modulators of GLUT4 translocation from a large number of antidiabetic plants.

1.3.2.1. *In vivo and in vitro* studies on antidiabetic plants.

Among the various plants studied for their antidiabetic effect, *Momordica charantia* is the one which is most important as it is a commonly used food material from the time immemorial. Various studies have proven the importance and antidiabetic effect of momordica in animal models and cell based systems. It is hypothesized that the
antidiabetic effect of momordica could involve a washout of glucose from the blood stream. Momordica fruit juice found to act like insulin in *in-vitro* cell based assays and proteins extracted from momordica fruit pulp was found to decrease plasma glucose concentrations in both normal and streptozotocin-induced diabetic rats and also found to induce glucose uptake in muscles and adipocytes [136, 137, 138, 139]. Apart from these, a study showed bioactive saponins in momordica fruit extract inhibits glucose uptake across the small intestine suggests its potential as an alternative drug therapy of postprandial hyperglycaemia [140]. Various other studies are also suggesting the *in-vivo* and *in-vitro* effect of momordica fruit extract on insulin secretion and glucose uptake [141, 142]. In addition to this a recent study had also shown the effect of triterpenoids isolated from bitter melon exerts its antidiabetic effect through the activation of AMPK pathway [143].

Similarly several other plant extracts are also shown to have antidiabetic effects either in animal models or in cell based systems. Studies on the antidiabetic effect of extracts of *Ginkgo biloba*, *Costus pictus*, *Agrimony eupatoria*, *Radix asparagi* and *Radix ophiopogonis* have showed that they stimulate glucose transport in various cell lines [144, 145, 146, 147, 148]. The aqueous extract of *Bauhinia megalandra* leaves and fenugreek were found to inhibit intestinal glucose absorption giving its importance in regulating the glucose absorption in to the blood from the intestine [149, 150]. Various extracts prepared from banaba (*Lagerstroemia speciosa L.*) [151], *Toona sinensis* [152], *Cinnamomum zeylanicum* [153, 154], *Costus afer* [155], *Amomi Semen* [156] and cinnamon are also found to enhance glucose transport in cultured adipocytes suggesting their potential antidiabetic effects. Similarly two important observations on *Pterocarpus marsupium* methanolic extract and fenugreek seed extract have shown the molecular mechanism their increased glucose transporter activity in various cell lines. The isolated isoflavone from *P. marsupium* was found to increase glucose transport in a PI3K independent mechanism and fenugreek was found to follow a wortmannin sensitive PI3K-PKC pathway without the involvement of protein kinase B [157, 158]. Methanolic extract of *Aegles marmelos*, *Syzygium cuminic* and *Canna indica* were also found to activate glucose transport in a PI3K dependent fashion but the active component was not isolated [159, 160].
Canadian lowbush blueberry (*Vaccinium angustifolium*) is another important traditional antidiabetic plant where the fermented juice of blueberry was found to increase glucose uptake in adipocyte and muscle in an insulin independent pathway through activating AMPK [161]. *Salacia oblonga* extract and mangiferin were also found to exert their antidiabetic effect by increasing GLUT4 expression and translocation in muscle cells in an AMPK mediated manner [162].

1.3.2.2. **Insulin secretagogue and insulin mimetic compounds purified from plant extracts**

Identification and characterization of active ingredient from the crude extract is very much important and studies have identified several compounds with antidiabetic activity *in-vivo* and *in-vitro*. Compounds like catechin derivatives, triterpenoids, triterpenoid saponins, sennosides, rhaponticin, maslinic acid and colosolic acid purified from various plant extract were found to have insulinogenic and insulin like properties suggesting their potential in antidiabetic treatment [163, 164, 165, 166, 167]. Various ellagitannins are also found to have insulin like glucose uptake stimulating activity in 3T3-L1 adipocytes [168, 169]. A homoisoflavone enriched fraction from *Liriope platyphylla* extract was found to increase GLUT4 translocation through increased insulin receptor substrate 1 (IRS1)-PI3 kinase-Akt signaling mechanism [170]. Purerarin isolated from the roots of *Pueraria lobata*, was found to enhance glucose uptake in C2C12 cells in a PLC-PKC (phospholipase C-protein kinase C) pathway [171].

Shikonin is another important compound that found to stimulate glucose uptake in 3T3-L1 adipocytes and its action was found to be inhibited by genistein and enhanced by vanadate indicating its modulatory effect on insulin signaling pathway [172]. Genistein, an isoflavonoid natural product, widely used to inhibit protein tyrosine kinase (PTK) was but found to enhance glucose uptake into C2C12 cells in a PLC dependent manner [173]. Steroidal glycosides and pentacyclic triterpinoides and synthesized derivatives of ursolic acid were found to enhance peripheral insulin sensitivity various cell based model systems [174, 175].
Flavonoids and flavonoid derivatives including the above discussed catechin derivatives form an important group of phytochemicals shown to have both stimulatory and inhibitory effect on glucose transport in various cell systems. Myricetin a flavonoid compound purified from *Abelmoschus moschatus* was found to decrease plasma glucose concentrations in a dose-dependent manner in STZ-diabetic rats suggesting its ability to enhance glucose utilization in diabetic rats lacking insulin [176]. Kaempferol and quercetin are generally considered as inhibitors of insulin signaling, however, same compounds isolated from Chinees folk medicine *Euonymus alatus* was found to act at multiple targets to ameliorate hyperglycemia [177]. Not only that, quercetin and quercetin 3-O-glycosides were found to be responsible for the antidiabetic activity of *Vaccinium vitis* berry extract that mediate through an AMPK signaling in C2C12 cells [178]. Similarly studies on a Korean antidiabetic plant showed the isolated triterpenes stimulate glucose uptake through increased phospho-AMPK, phospho-ACC, and phospho-GSK-3beta activation in a concentration dependent manner [179]. Lupinoside, aspalathin, 3',5'-diprenylgenistein, 6,8-diprenylgenistein, derrone and alpinumisoflavone from various plant extracts were found to increase glucose uptake in various cells lines. Lupinoside found to increase glucose uptake in insulin resistant adipocytes suggesting its role in antidiabetic therapy [180, 181, 182].

### 1.3.3. Target based therapeutic approach for insulin resistance

Eventhough insulin mediated glucose disposal is the key mechanism that regulates blood glucose homeostasis; several other biochemical and physiological events are also considered important for this regulation. Regulation of digested carbohydrate absorption and regulation of post-absorption biochemical pathways like glycolysis, krebs cycle, glucosneogenisis, glycogen synthesis and glycogenolysis are also shown to be important as targets for the treatment of diabetes. Various plants have shown to exert their antidiabetic effect through regulating these pathways [183, 184, 185, 186]. However, since insulin resistance is a characteristic phenomenon in type 2 diabetes, most of the antidiabetic therapeutic approaches focus on counteracting insulin resistance in peripheral tissues using various treatment modalities. As discussed earlier, in insulin resistance, despite the presence of sufficient amount of insulin in the body, a failure in insulin action occurs resulting in decreased glucose uptake in to the peripheral tissues due to reduced
GLTU4 translocation to the plasma membrane. Hence GLUT4 become an important target for the treatment of insulin resistance and type 2 diabetes and there has been considerable interest in identifying new compounds that can increase GLUT4 translocation in adipocytes and muscle.

1.4. Significance of the present study

Type 2 diabetes, a disease reaching epidemic status worldwide, is associated with microvascular and macrovascular complications which are the major causes of mortality associated with this disease. On a molecular level, insulin resistance, characteristic feature of type 2 diabetes, is associated with impaired translocation of GLUT4 to the plasma membrane and reduced glucose disposal leading to hyperglycemia. This finding emphasizes the importance of GLUT4 as a drug target for type 2 diabetes. Any compound that increases the exocytosis and/or decreases the endocytosis will result in increased GLUT4 expression on plasma membrane and ultimately enhances glucose absorption. The present research thesis focuses on identification and characterization of compounds from medicinal plants that can modulate GLUT4 translocation and elucidating the molecular mechanism underlying the process. Such study would lead to the identification of novel therapeutic target.