Introduction
Glucose Homeostasis

Energy metabolism and homeostasis are among the most imperative biological processes to the survival of a living organism. Energy homeostasis is governed by the consumption, storage and utilization of substrates to drive the reactions necessary for normal physiological function (Kelley and Mandarino, 2000; Storlien et al., 2004). Glucose and lipids are the key substrates in energy balance and their metabolism is closely regulated in human physiology. The human body needs to be well adapted to cope with major challenges in the supply and demand of energy.

Glucose is the most common substrate for energy metabolism. Certainly, under normal circumstances glucose is the only form of energy that can be used by the brain and central nervous system (Pellerin and Magistretti, 2003; Thorens and Mueckler, 2010). The brain cannot synthesize glucose or store glycogen; it is critically dependent on a continuous supply of glucose from the circulation (Boyle et al., 1995; Berg et al., 2002; Ohtsuki, 2004; Vijayakumar et al., 2012). When glucose levels fall, blood-to-brain glucose transport becomes limiting to brain glucose metabolism, and ultimately survival (Cryer, 2001; Cryer et al., 2003; Bree et al., 2009). The amount of glucose in the circulation is dependent upon a balance of the rate of glucose entering the circulation (glucose appearance) and the rate of glucose removal from the circulation (glucose disappearance) (Gerich, 2000). Circulating plasma glucose is derived from intestinal absorption, glycogenolysis, the breakdown of glycogen, which is the storage form of glucose, and gluconeogenesis, the formation of glucose primarily from lactate, glycerol and amino acids during the fasting state (Saltiel and Kahn, 2001). Despite the varying levels of glucose after a meal or during fasting and exercise, the body needs to regulate its internal environment and maintain a constant and stable condition (Vidal-Puig and O’Rahilly, 2001). Hence, blood glucose levels are under the regulation of various homeostatic
control mechanisms which maintain them within a narrow range in both the fasting and feeding states.

**Glucoregulatory hormones**

Glucose is maintained at normoglycemic condition by interplay of various hormones (MacLeod, 2000; Hayes, 2008). The principal level of control on glycaemia by the islet of Langerhans depends largely on the coordinated secretion of glucagon and insulin by α- and β-cells, respectively. Both cell types respond oppositely to changes in blood glucose concentration (Quesada et al., 2008). The glucoregulatory hormones in glucose homeostasis require a harmonized interaction between several tissues, achieving equilibrium between glucose output and uptake (Unger, 1966; Nadal et al., 1999; Quesada et al., 2006).

**Insulin**

Insulin, a small protein composed of two polypeptide chains containing 51 amino acids, is a key anabolic hormone. Insulin was the only pancreatic β-cell hormone known to lower blood glucose concentrations. The storage and release of energy during feeding and fasting or for the maintenance of cellular growth and survival are tightly regulated by the insulin signalling system. Insulin exerts its actions through binding to its specific receptor present on many cells of the body, including fat, liver, and muscle cells. Insulin is the major regulator of glucose metabolism, although its actions are modified in many respects by counter-regulatory hormones (glucagon, adrenaline, cortisol and growth hormone) (Aronoff et al., 2004; Wilcox, 2005; Varewijck and Janssen, 2012). Insulin is secreted by the pancreatic β-cells in response to elevated levels of circulating levels of glucose and amino acids after a meal (Sesti, 2006). At the molecular level, interaction of the alpha and beta subunits of the receptor followed by insulin binding results in activation of tyrosine kinase activity, which is integral to the initiation of cascade of phosphorylation/dephosphorylation reactions and
translocation of glucose transporter (GLUT) to the plasma membrane that mediate actions of insulin that ultimately lead to modifications in a number of biological processes (Purrello et al., 1983; Kahn and Folli, 1993; Saltiel, 1994; Saltiel, 1996). To maintain circulating glucose concentrations in a relatively narrow range, insulin decreases blood glucose levels by suppressing hepatic glucose output and facilitating glucose uptake in liver, extrahepatic tissues (muscle and adipose tissue), glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycemia (Rhodes and White, 2002; MacDonald et al., 2005; Edgerton et al., 2008; Brady, 2010; Manning, 2010). Other actions of insulin include stimulation of fat synthesis, promotion of triglyceride storage in fat cells, promotion of protein synthesis in the liver and muscle, and proliferation of cells (Hill and Milner, 1985; Cryer, 1992; Kahn and Flier, 2000; Rossi and Vergnanini, 2000; Saltiel and Kahn, 2001; Guilherme et al., 2008). This regulation depends on the state of the body and can be modified by counter-regulatory hormones, which increase blood glucose concentrations. The effect of counter-regulatory hormones is to cause greater production of glucose from the liver and less utilization of glucose in adipose and muscle tissues (Cryer, 1992; Gerich, 1993; Aronoff et al., 2004). During fasting, the liver produces glucose both by glycogenolysis and gluconeogenesis, whereas in the fed state the liver takes up glucose and stores glucose as glycogen. In skeletal muscle and adipose tissue mainly insulin increases the rate of glucose uptake (Roy et al., 1998; DeFronzo and Tripathy, 2009). Skeletal muscle, due to its large mass, is the principal organ for glucose disposal in the body (DeFronzo et al., 1985).

**Glucagon**

Glucagon is a key catabolic hormone consisting of 29 amino acids. Glucagon, being the biologic antagonist of insulin, counteracts the effects of insulin so that fluctuations in blood glucose levels are prevented (Unger and Orci, 1981; Petersen et al., 1987; Quesada et al., 2008). Administration of exogenous glucagon increases glucose level in fasted or fed
animals and similar observations were made in humans (Lins et al., 1983; Freychet et al., 1988; Myers et al., 1991; Young et al., 1993; Hvidberg et al., 1994). Small doses of glucagon are sufficient to induce significant glucose elevations. Several lines of evidence indicate that glucagon is a sensitive and timely regulator of glucose homeostasis in vivo (Lins et al., 1983; Myers et al., 1991; Haymond and Schreiner, 2001). It is secreted from pancreatic α-cells. Hepatic glucose production, which is primarily regulated by glucagon, maintains basal blood glucose concentrations within a normal range during the fasting state. When plasma glucose falls below the normal range, glucagon secretion increases, resulting in hepatic glucose production and return of plasma glucose to the normal range (Orci et al., 1975; Gerich et al., 1979; Aronoff et al., 2004).

The binding of glucagon to the extracellular loops of its receptor results in conformational changes of the latter, leading to subsequent activation of the coupled G-proteins. When Ga is activated, it in turn activates adenylate cyclase, which increases PKA through cAMP. This increases peroxisome proliferator-activated receptor-γ coactivator-1 alpha (PGC-1α), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) triggering gluconeogenesis (Beale et al., 1984; Iynedjian et al., 1985; Christ et al., 1988; Jiang and Zhang, 2003). In a subsequent pathway, when the Gq is activated it results in the activation of phospholipase C, production of inositol 1,4,5-triphosphate, and subsequent release of intracellular calcium which ultimately decreases glycolysis and glycogenesis and increases gluconeogenesis (Christophe, 1995; Burcelin et al., 1996; Jiang and Zhang, 2003).

Hepatic glucose production maintains basal blood glucose concentrations within a normal range during the fasting state (Sherwin et al., 1980; Hellerstein et al., 1997; Xu et al., 2011). Glucagon signalling promotes glycogenolysis and, at the same time, inhibits glycogen synthesis in the liver. Glucagon stimulation, activates PKA which phosphorylates and activates glycogen phosphorylase kinase. This activated kinase subsequently phosphorylates
glycogen phosphorylase at serine-14 residue leading to its activation. Further, activated glycogen phosphorylase favours the phosphorylation of glycogen, resulting in increased glycogen breakdown (glycogenolysis) and the production of glucose 6-phosphate (G-6-P). G-6-P is then converted into glucose by glucose-6-phosphatase (G-6-Pase), increasing the glucose pool for hepatic output. In addition to that glucagon has been shown to increase G-6-Pase activity (Band and Jones, 1980a; Band and Jones, 1980b; Krebs, 1981; Striffler et al., 1984; Johnson et al., 1997).

This endogenous source of glucose is not needed during and immediately following a meal, and glucagon secretion is suppressed then. When coupled with insulin’s direct effect on the liver, glucagon suppression results in a near-total suppression of hepatic glucose output. In the diabetic state, there is inadequate suppression of postprandial glucagon secretion (Cryer, 1981; Dinneen et al., 1995) resulting in elevated hepatic glucose production. Importantly, exogenously administered insulin is unable both to restore normal postprandial insulin concentrations in the portal vein and to suppress glucagon secretion through a paracrine effect. This results in an abnormally high glucagon-to-insulin ratio that favours the release of hepatic glucose (Baron et al., 1987; Aronoff et al., 2004).

**Amylin**

Islet amyloid polypeptide (IAPP), also called amylin, a 37-amino acid polypeptide, has been localized in the β-cell secretory granule and is co-secreted with insulin (Dégano et al., 1993). Amylin is released along with insulin from beta cells. It decreases glucagon level, which will then decrease the hepatic glucose production. The overall effect of the hormone is to reduce the production of sugar by the liver during a meal to prevent it from getting too high (Young et al., 1990; Schmitz et al., 2004).
Catecholamines

Catecholamines, which include dopamine, noradrenaline, and adrenaline, are important neurotransmitters and hormones that regulate visceral functions, motor coordination and arousal in adults (Zhou et al., 1995; Britsch et al., 1998). Catecholamines can exert their effects on carbohydrate metabolism directly, through stimulation of adrenoreceptors and indirectly, mainly through modulation of pancreatic insulin and glucagon release. Both direct and indirect effects result in a prompt rise in plasma glucose (Bearn et al., 1951; Clutter et al., 1988; Diamanti-Kandarakis et al., 2003). Under physiologic conditions, infusing catecholamine is associated with enhanced rates of aerobic glycolysis (resulting in ATP production), glucose release (both from glycogenolysis and gluconeogenesis), and inhibition of insulin-mediated glycogenesis consequently resulting in hyperglycemia (Rizza et al., 1979; Halter et al., 1984; Barth et al., 2007; Kotulak et al., 2010; Weil, 2012).

Growth hormone

The insulin antagonistic effects of growth hormone (GH) include increased blood glucose concentration and inhibition of glucose transport (Davidson, 1987; Møller et al., 1991; Dominici and Turyn, 2002), which has been observed in lower animals under conditions of excessive endogenous GH production, after administration of exogenous GH, as well as in transgenic mice and rabbits over expressing GH (Dominici and Turyn, 2002). The chronic GH excess is generally associated with reduced levels of insulin receptor and its phosphorylation in skeletal muscle and liver of mice and rat (Smith et al., 1997; Thirone et al., 1998). Further, insulin-stimulated p85 subunit of PI3K-IRS-1/IRS-2 association, as well as the insulin-stimulated PI3 kinase activity was also impaired. However, GH deficiency in human is associated with increased insulin sensitivity, decreased insulin secretion, and decreased fasting glucose concentration (Hopwood et al., 1975; Bougneres et al., 1985; Jorgensen et al., 2006).
**Glucocorticoids**

Glucocorticoids are steroid hormones that regulate multiple aspects of glucose homeostasis. Glucocorticoids (primarily cortisol in humans) are released from the adrenal cortex in response to stress; one such stress is a decrease in plasma glucose (Pilkis et al., 1988; Macfarlane et al., 2008). Glucocorticoids promote gluconeogenesis in liver, whereas in skeletal muscle and white adipose tissue they decrease glucose uptake and utilization by antagonizing insulin response (Wang and Harris, 2015). Therefore, excess glucocorticoid exposure causes hyperglycemia and insulin resistance. Glucocorticoids also regulate glycogen metabolism (Macfarlane et al., 2008; Wang and Harris, 2015). In liver, glucocorticoids increase glycogen storage, whereas in skeletal muscle they play a permissive role for catecholamine-induced glycogenolysis and/or inhibit insulin-stimulated glycogen synthesis. Moreover, glucocorticoids modulate the function of pancreatic α and β cells to regulate the secretion of glucagon and insulin, two hormones that play a pivotal role in the regulation of blood glucose level. Overall, the major glucocorticoid effect on glucose homeostasis is to preserve plasma glucose for brain during stress, as transiently raising blood glucose is important to promote maximal brain function (Di Dalmazi et al., 2012; Kuo et al., 2015).

Normally in response to insulin the liver decreases its output of glucose. Glucocorticoids decrease the liver sensitivity to insulin, thereby increasing hepatic glucose output. They also inhibit glucose uptake in muscle and fat, reducing insulin sensitivity as much as 60% in healthy volunteers. This seems primarily due to a post receptor effect, i.e. inhibition of glucose transport (Lansang and Hustak, 2011).

**Sex steroids**

High circulating levels of sex steroids appear to contribute for the development of insulin resistance and women with low serum levels of sex steroids or high serum testosterone
are at greater risk of developing type-2 diabetes (Bruns and Kemnitz, 2004; Rao et al., 2013; Vejrazkova et al., 2014). Peripheral action of sex steroids and the fact that skeletal muscles are responsible for the majority of peripheral glucose disposal, tempt to propose that sex steroids have a direct action on skeletal muscles to modulate glucose homeostasis (Sato and Iemitsu, 2015). Administration of testosterone or its derivatives to women resulted in impaired glucose tolerance and hyperinsulinemia, indicative of insulin resistance (Landon et al., 1963; Diamond et al., 1998). There is extensive experimental evidence that sex steroids and insulin interact in their action on target tissues (Livingstone and Collison, 2002). At physiological level, testosterone and estradiol are involved in maintaining normal insulin sensitivity. However, outside the ‘physiological window’ these steroids may promote insulin resistance (Livingstone and Collison, 2002; Kapoor et al., 2006; Muthusamy et al., 2007).

**Role of skeletal muscle in glucose homeostasis**

There are two classes of muscles, smooth and striated; striated muscle is further divided into heart and skeletal muscle. Smooth muscle surrounds blood vessels and the gastrointestinal tract (Borg and Caulfield, 1980). Skeletal muscle is composed of both oxidative (slow-twitch) and glycolytic (fast-twitch) fibers (Ariano et al., 1973).

Skeletal muscle is one of the major consumers of circulating fuels (glucose, lipids) in the body partly because of its large mass as compared to other tissues, and partly due to higher metabolic demands during strenuous activities. Skeletal muscle is a major site of substrate metabolism and responsible for insulin- and exercise-mediated glucose disposal (DeFronzo et al., 1985). Skeletal muscle is quantitatively the most important tissue for glucose and lipid metabolism and although adipose tissue is important in the storage of lipids, skeletal muscle is the largest consumer of lipids as an energy substrate. The tissue depends upon the catabolism of lipids during periods of low glucose availability. Under the
euglycemic hyperinsulinemic clamp, skeletal muscle accounts for 80-90% of glucose disposal (Dagenais et al., 1976; DeFronzo et al., 1981a). Thus, cellular mechanisms regulating glucose uptake in skeletal muscle have a major impact on whole-body glucose homeostasis. The transport of glucose into skeletal muscles is the initial, and under many physiological conditions the rate-limiting event in glucose metabolism and a site of insulin resistance in patients with type-2 diabetes mellitus (DeFronzo et al., 1992; Shulman, 2000).

Glucose transporter protein isoforms differ in properties such as their maximal rates of glucose transport, their ability to bind glucose, and the regulation of their activity by various hormones (Bell et al., 1990; Mueckler, 1990). Insulin increases glucose transport in adipocytes and myocytes by stimulating the translocation of the glucose transporter 4 (GLUT4) from an intracellular pool to the plasma membrane. There are many potential steps at which GLUT4 translocation could be impaired in insulin-resistant subjects, including upstream insulin signalling events (Gumà et al., 1995; Sesti, 2006). Storage of glucose as glycogen is enhanced by insulin stimulation of glucose uptake in muscle, via increased translocation of intracellular vesicles containing GLUT4 to the cell surface (Ryder et al., 2001; Jessen and Goodyear, 2005). Under fasting conditions, plasma glucose and insulin concentrations are low, leading to an increase in non-esterified fatty acid (NEFA) concentrations; thus fatty acids are then the main energy substrate for skeletal muscle (Tremblay et al., 2003). During exercise or muscle contraction, glucose uptake increases, as a result of activation of AMP-activated protein kinase (AMPK) as well as increased intracellular Ca^{2+} levels (Hutchinson et al., 2008). The latter is also able to stimulate glucose uptake independently of contraction (Youn et al., 1991).

Skeletal muscle insulin resistance is considered to be the initiating or primary defect that is evident decades before beta-cell failure and overt hyperglycaemia develops (DeFronzo, 2009). Binding of insulin to its receptor (insulin receptor) activates the receptor tyrosine
kinase, which phosphorylates and recruits the insulin receptor substrate (IRS) family of proteins. Tyrosine-phosphorylated IRS display binding sites for phosphatidylinositol-3 kinase (PI3K), which, in turn, activates Akt/protein kinase B, resulting in increased translocation of intracellular stored glucose transporter 4 (GLUT4) subtype to the plasma membrane (Watson and Pessin, 2001; Saltiel and Pessin, 2002). The activation of IRS-PI3K-Akt pathway facilitates glucose uptake by the skeletal muscle cells. In insulin resistant states such as obesity, hypertension, and type-2 diabetes, insulin-induced glucose transport is markedly decreased in skeletal muscle, due to an impaired expression and functionality of the insulin signalling pathway (Henriksen and Prasannarong, 2013). Skeletal muscle is also a primary site of insulin resistance in the context of metabolic disease, in particular, type-2 diabetes and obesity (Bouzakri et al., 2005).

**Diabetes Mellitus**

Diabetes mellitus refers to a group of syndromes characterized by hyperglycemia, i.e. abnormally high blood glucose levels. This hyperglycemia resulting from defects in insulin secretion or action or both. Several pathogenic processes are involved ranging from autoimmune destruction of the β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action (Aronoff et al., 2004). The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and / or diminished tissue responses to insulin at one or more points in the complex pathway of hormone action (Gupta et al., 2014; International Diabetes Federation (IDF), 2015). There are several less common forms of diabetes (e.g. gestational diabetes) but the most common variants are type-1 and type-2, where the latter stands for the vast majority (roughly 90%) of the cases (Scully,
The dramatic increase in obesity and diabetes worldwide poses a huge socioeconomic burden to healthcare system.

**Type-1 diabetes mellitus**

Type-1 diabetes (T1DM) is an autoimmune disorder characterized by organ-specific immune destruction of insulin-producing pancreatic β-cells in the islets of Langerhans within the pancreas (Wilcox et al., 2016). Type-1 diabetes represents one of more than 80 diseases considered to have an autoimmune aetiology. Once diagnosed, patients require lifelong insulin treatment and can experience numerous disease-associated complications. Type-1 diabetes is usually diagnosed in children and young adults, and was previously known as juvenile diabetes. Only 10% of people with diabetes have this form of the disease (Bluestone et al., 2010).

The β-cells are elegant glucose ‘thermostat’, sensing glucose and releasing insulin to maintain physiologic glucose levels within a relatively narrow range. They thus comprise much more than just an insulin factory. Once these cells are destroyed, patients with type-1 diabetes lose blood glucose control, which can result in both acute conditions (for example, ketoacidosis and severe hypoglycaemia) (Vauzelle-Kervroëdan et al., 1999) and secondary complications (including heart disease, blindness and kidney failure) - even with current insulin replacement therapies (Maahs and Rewers, 2006).

**Type-2 diabetes mellitus**

Type-2 diabetes mellitus, also known as adult-onset diabetes, maturity-onset diabetes (Tuomilehto et al., 2001; Ndisang et al., 2015). In type-2 diabetes, a combination of peripheral insulin resistance and aberrant production of insulin are amongst the paradox commonly encountered in the pathogenesis of the disease. The state in which insulin resistant individuals are unable to secrete enough insulin to overcome the defect in insulin signalling,
leads to development of type-2 diabetes (Ndisang et al., 2015). Insulin resistance has traditionally been associated with type-2 diabetes, and recent evidence suggests that insulin resistance in type-1 diabetes is increasing (Nokoff et al., 2012; Cleland et al., 2013; Polsky and Ellis, 2015; Bacha and Klinepeter Bartz, 2016). The sedentary lifestyle and energy-rich diet in the present industrialized world is aggressively contributing to a staggering increase in obesity resulting in insulin resistance. As a result, there is a steady increase in the pathogenesis of type-2 diabetes. It accounts for 90-95% in total diabetic population (Wilmot et al., 2012; Rockette-Wagner et al., 2015).

The etiology of insulin resistance is complicated and several factors are implicated, so deciphering this multifaceted disease remains challenging, although a wide body of evidence suggests that oxidative stress, inflammation, genetic, habitual, environmental, and epigenetic factors may be involved (Slomko et al., 2012; Wang et al., 2015b). Several lines of evidence show that genetic susceptibility for type-2 diabetes could also induce an early β-cell dysfunction, leading to progression of type-2 diabetes (Prentki and Nolan, 2006; Muoio and Newgard, 2008). It is primarily represented by decreased insulin-stimulated glucose uptake in skeletal muscle, unsuppressed hepatic glucose production, and increased lipolytic activity in adipose tissue (DeFronzo, 2004). In addition to elevated blood glucose levels, type-2 diabetic patients are characterized by a decreased fat oxidative capacity and high levels of circulating free fatty acids (FFAs) (Kelley et al., 1999).

Type-2 diabetes is associated with impaired “metabolic flexibility” i.e. an impaired switch from fat to glucose oxidation in response to insulin (Kelley, 2005). Thus, a reduced ability to oxidize lipids and/or metabolic inflexibility, are important components of skeletal muscle insulin resistance (Phielix and Mensink, 2008). Insulin resistance as well as a cluster of risk factors which often coexist, including high plasma triglycerides, low high-density lipoprotein cholesterol and essential hypertension, together increase the risk of cardiovascular
disease (Zhang et al., 2010). This condition has been termed the ‘metabolic syndrome’, and insulin resistance per se has been postulated as an underlying mechanism (Kahn et al., 2014; Kaur, 2014). Given the current world wide increase in type-2 diabetes prevalence, investigations into the underlying mechanisms of this disorder continue to be important.

**Gestational diabetes mellitus**

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (American Diabetes Association (ADA), 2016). Gestational diabetes affects around 7% of pregnant women, however the value of screening women for gestational diabetes has been hotly debated. Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester. The condition arises because the action of insulin is blocked, probably by hormones produced by the placenta (International Diabetes Federation (IDF), 2013). Gestational diabetes is associated with substantial rates of maternal and perinatal complications (Zhang et al., 2015). Poorly managed blood glucose during pregnancy can lead to a significantly larger than average baby (a condition known as fetal macrosomia), which makes a normal birth difficult and risky (Najafian and Cheraghi, 2012). Long-term adverse health outcomes reported among infants born to mothers with gestational diabetes include sustained impairment of glucose tolerance (Silverman et al., 1995), subsequent obesity (Petitt et al., 1985) and impaired intellectual achievement (Rizzo et al., 1997). Women who have had gestational diabetes are at a higher risk of developing gestational diabetes in subsequent pregnancies and of developing type-2 diabetes later in life (Henry and Beischer, 1991).

**Insulin signalling**

The driving force that regulates is insulin, in response to elevated levels of nutrients, such as glucose in the blood supply (Lizcano and Alessi, 2002). When insulin binds to its
receptor, resultant activation of the insulin signaling cascade leads to multiple effects on several biological processes, including glucose and lipid uptake/metabolism, gene expression/protein synthesis and cell growth, division and survival (Rhodes and White, 2002). Normal glucose regulation is achieved by having adequate insulin secretion and effective glucose uptake/disposal (Kutyavin and Chawla, 2016).

**Molecules of insulin signalling cascade**

**Insulin receptor (IR)**

Insulin receptor is a member of the ligand-activated receptor and tyrosine kinase family of transmembrane signalling proteins that are fundamentally important regulators of cell differentiation, growth, and metabolism (Lee and Pilch, 1994). Tyrosine phosphorylated residues on the receptor itself and on subsequently bound receptor substrates provide docking sites for downstream signalling molecules, including adapters, protein serine/threonine kinases and exchange factors including the IRS proteins and members of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway resulting in translocation of glucose transporters to the cell surface (Pirola et al., 2004; Bhaskar et al., 2012). The insulin receptor (IR) is present in all vertebrate tissues with the highest concentration in the major metabolic organs such as muscle, adipose tissue and liver (Youngren, 2007). The IR is a disulfide-linked protein composed of two extracellular α (contains ligand-binding domain) and two intrinsic β subunits (contains the tyrosine kinase signalling domains) present in the plasma membrane of target cells (Tiwari et al., 2007). Alterations in the expression levels of the IR and IRS-1 have been reported in the onset of type-2 diabetes in animal models and humans (Saad et al., 1992; Goodyear et al., 1995; Zierath et al., 2000; Choi and Kim, 2010; Galkina et al., 2012). IR knockout mice are slightly small at birth and die after a few days, owing to extreme hyperglycemia and ketoacidosis (Long et al., 2011).
Insulin receptor substrate (IRS)

Insulin receptors (IR) belong to the receptor tyrosine kinase (RTK) superfamily, signal transmission by these receptors mainly occurs via the insulin receptor substrate (IRS) adapter proteins. These adapter proteins bind to the transphosphorylated activated receptors at tyrosine docking sites, are themselves phosphorylated, and in turn recruit SH2 domain-containing signalling molecules to form the productive signalling complex (Gual et al., 2005).

Several substrates have been identified for the insulin receptor, including the insulin receptor substrate proteins, Shc, Gab-1, Cbl and APS (Saltiel and Pessin, 2002). The IRS proteins do not contain any intrinsic kinase activity, but rather serve as scaffolds to organize signalling complexes and initiate intracellular signalling pathways (Mardilovich et al., 2009). IRS1 contains pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains at the N-terminus which couple IRS1 to phosphorylated IR (Gual et al., 2005).

IRS1 becomes tyrosine phosphorylated and recruits a number of SH2 containing signal transducers including PI3-kinase (Sesti et al., 2001; White, 2002). The phosphorylation of Tyr608 and Tyr628, for instance, generates the major docking sites for the PI 3-kinase (Esposito et al., 2001). Phosphorylation of IRS proteins by insulin receptor, lead to the activation of two main signalling pathways: the PI3K-Akt/PKB pathway, which are responsible for most of the metabolic actions of insulin, and the Ras-MAPK pathway, which regulates expression of some genes and co-operates with the PI3K pathway to control cell growth and differentiation (Yamauchi et al., 1996; Taniguchi et al., 2006). Dysfunction of IRS proteins initially leads to post-prandial hyperglycemia, increased hepatic glucose production, and dysregulated lipid synthesis and is discussed as major pathophysiological mechanism for the development of insulin resistance and type-2 diabetes mellitus (Fritsche et al., 2008).
Phosphatidylinositol-3-kinase (PI3K)

IRS1 and IRS2 appear to be the adapter molecules playing a major role in the coupling to PI3K-PKB and MAPK downstream kinases (Frojdo et al., 2009). PI3K is a heterodimeric lipid kinase (consisting of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit) that phosphorylates the D-3 position on the inositol ring in phosphoinositides (Riley et al., 2006) thereby converting PIP2 to PIP3 (Pollheimer and Knöfler, 2005). PI3-kinase catalyzes the formation of lipid second messenger phosphatidylinositol-3,4,5 triphosphate [PI(3,4,5)P3], which is necessary to recruit downstream kinases, such as Phosphatidylinositol-dependent protein kinase 1 (PDK-1) and Akt (also called protein kinase B; PKB) (Copps and White, 2012). Gene knockout of the catalytic subunit results in insulin resistance and glucose intolerance (Boucher et al., 2014). Collectively, these studies demonstrate that PI 3-kinase is required for insulin-stimulated glucose transport (Ueki et al., 2002; Cahill et al., 2012).

PKB/Akt

Protein kinase B or Akt (PKB/Akt) is a serine/threonine kinase, which in mammals comprises three highly homologous members known as PKBalpha (Akt1), PKBbeta (Akt2), and PKBgamma (Akt3) (Nicholson and Anderson, 2002). Akt1 is the major isoform ubiquitously expressed, while Akt2 is less abundant, except in insulin responsive tissues (Altomare et al., 1995; Altomare et al., 1998; Hanada et al., 2004). The third isoform Akt3 has been described mostly in brain, testis and beta-cells (Holst et al., 1998). The activity of Akt is induced following PI3-kinase activation in response to treatment with a wide variety of growth stimuli, including insulin, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF) (Burgering and Coffer, 1995; Franke et al., 1995). PIP3 recruits Akt from the cytosol to the plasma membrane where it is activated by other kinases such as phosphoPhosphatidylinositol-dependent protein kinase 1 (PDK1).
which are co-localized along with Akt at PIP3 (Carnero et al., 2008). PDK1 phosphorylates Akt at threonine 308 (Thr308) which is followed by a second phosphorylation event by the mammalian target of rapamycin C2 (mTORC2) on Akt at serine 473 (Ser473), which potentiates the Akt kinase activity (Dunn and Connor, 2011). Full activation of Akt requires phosphorylation on two major sites, Thr308 and Ser473, and phosphorylation on these two sites are PI3-kinase dependent (Alessi et al., 1996). It has been shown that mutation of these two sites to alanine (T308A/S473A) inhibits Akt activation, whereas mutation to acidic residues (T308D/473D) renders the kinase constitutively active (Bellacosa et al., 1998). Studies have suggested that inhibition of Akt activity may be the primary site for impaired insulin signalling in skeletal muscle (Storz et al., 1999; Arora, 2010; Sylow et al., 2014).

β-Arrestin-2

β-arrestin-2 was originally identified as a mediator of β-adrenergic receptor endocytosis, leading to attenuation of β-adrenergic signalling. They regulate the desensitization and downregulation of many G protein-coupled receptors (GPCRs) (Lefkowitz et al., 2006; Ahn et al., 2013). It is reported that β-arrestin-2 regulates insulin action by scaffolding a complex containing insulin receptor, c-Src, and Akt (Kovacs et al., 2009). This complex allows c-Src to phosphorylate Akt at Tyr315 and Tyr326, which are required for the subsequent phosphorylation of Akt at Thr308 and Ser473 by PDK1 and PDK2, respectively (Stöckli and James, 2009). Insulin dependent Akt tyrosine phosphorylation was defective in β-arrestin-2−/− mice, concomitant with reduced phosphorylation of Akt at Thr308 and Ser473, the two sites that are essential for full Akt activation (Stöckli and James, 2009). Further, it is also stated that the disruption in the formation of this complex, due to reduced β-arrestin-2 expression, leads to impaired Akt activation followed by insulin resistance (Luan et al., 2009).
Akt substrate of 160 kDa (AS160)

AS160 is described as substrate for the protein kinase Akt that links insulin signalling and GLUT4 trafficking. AS160, has been shown to be phosphorylated on threonine residues in response to insulin that conforms to the requirements of Akt target motifs (Bouzakri et al., 2008). This molecule contains two phosphotyrosine binding (PTB) domains at the NH2 terminus and a Rab GTPase activating protein (GAP) domain at the COOH terminus (Tan et al., 2012a). AS160 is a molecular link among diverse signalling cascades such as activation of conventional/novel protein kinase C (n/cPKC), 5′ AMP-activated protein kinase (AMPK) and platelet-derived growth factor (PDGF) converging on GLUT4 translocation (Thong et al., 2007). Insulin-stimulated phosphorylation of the Akt substrate AS160 is impaired in skeletal muscle of type-2 diabetic subjects (Karlsson et al., 2005). A single mutation of AS160-Thr642 to Ala reduced the insulin-stimulated GLUT4 translocation by $\sim60\%$ and it is reported that phosphorylation of AS160 is required for the insulin-induced translocation of GLUT4 to the plasma membrane in 3T3L1 adipocytes (Sano et al., 2003). In skeletal muscle and adipocytes, at physiological conditions, AS160 is bound to GLUT4 but, once Akt phosphorylates AS160 through insulin signalling, the GLUT4 vesicles are unrestricted to move to the plasma membrane (Karlsson et al., 2005; Thong et al., 2007; Tan et al., 2012b).

Glucose Transporters

Glucose is a polar molecule and does not readily diffuse across the hydrophobic plasma membrane. The uptake of glucose into tissues involves specific carrier molecules known as Glucose Transporters (GLUTs) (Czech and Corvera, 1999). These transporters contain 12-transmembrane domains, and they facilitate the diffusion of glucose down a concentration gradient in an energy-independent manner. There are 13 members of the GLUT
family which can be divided into three subclasses based on their conserved structural characteristics (Joost et al., 2002).

Among the three subclasses, Class I GLUTs, which comprise of GLUTs1-4, are the best characterised transporters of the family. GLUT1 is expressed in most tissues although it is quite abundant in brain and erythrocytes (Gould et al., 1991), with moderate levels of expression in adipose tissue, muscle, and the liver. It appears to provide a low, constitutive level of glucose transport required for basal cellular processes, probably in combination with several other GLUT isoforms (Mueckler et al., 1997). GLUT2 is found in liver cells, pancreatic β-cells, small intestine, and kidney. It has a low affinity for glucose and functions as part of the glucose sensor system in β-cells and also regulates absorption of glucose in intestinal and renal epithelial cells (Fukumoto et al., 1988). GLUT3 has the highest affinity for glucose. It is expressed primarily in neuronal tissue and is considered the major GLUT responsible for transporting glucose into the central and peripheral nerves (Mantych et al., 1992). In contrast, GLUT4 is the main insulin-responsive glucose transporter and is predominantly expressed in skeletal and cardiac muscles, and in brown and white adipose tissues (James et al., 1988).

**Rab proteins**

Rab proteins constitute the largest branch of the Ras GTPase superfamily. Rabs use the guanine nucleotide-dependent switch mechanism, where they function as regulators of distinct steps in membrane traffic pathways (Stenmark and Olkkonen, 2001). They regulate each of the four major steps in membrane traffic pathways such as vesicle budding, vesicle delivery, vesicle tethering, and fusion of the vesicle membrane with that of the target compartment and these different tasks are carried out by a diverse collection of effector molecules that bind to specific Rabs in their GTP-bound state (Grosshans et al., 2006). Rab
GTPases function as molecular switches, cycling between GTP-bound and GDP-bound states. This switch is controlled by guanine nucleotide exchange factors (GEFs), which trigger the binding of GTP, and GTPase-activating proteins (GAPs), which accelerate hydrolysis of the bound GTP to GDP (Pfeffer and Aivazian, 2004).

**IRAP protein**

Insulin-regulated aminopeptidase (IRAP) is an abundant cargo protein of GLUT4 storage vesicles (GSVs) that traffics to and from the plasma membrane in response to insulin (Kandror and Pilch, 1994). In the basal state, most GSVs are sequestered in perinuclear and other cytosolic compartments (Ross et al., 1997). In response to insulin, GSVs undergo robust exocytosis to deliver cargo proteins to the plasma membrane, enabling GLUT4 to import glucose and IRAP to proteolyse specific circulating hormones (Keller, 2003). Insulin-stimulated GLUT4 translocation was impaired upon IRAP knockdown, indicating that IRAP plays a role in GSV trafficking (Bryant et al., 2002). Insulin modulates the exocytosis of diverse membrane proteins from the endosomal compartments to the plasma membrane. This effect is most prominent on the glucose transporter GLUT4 and IRAP (Keller, 2003).

**VAMP protein**

Insulin stimulates glucose transport in skeletal muscle, heart, and adipose tissue by promoting the appearance of GLUT4, the major glucose transporter isoform present in these tissues, on the cell surface. This is achieved by differentially modulating GLUT4 exocytosis and endocytosis, between a specialized intracellular compartment and the plasma membrane (St-Denis and Cushman, 1998). Vesicle-associated membrane proteins (VAMP) are a family of SNARE (soluble NSF attachment protein receptors where NSF stands for N-ethylmaleimide-sensitive fusion protein) proteins with similar structure, and are mostly involved in vesicle fusion. v-SNARE (vesicle-associated proteins) proteins, such as the
vesicle-associated membrane proteins (VAMPs) form high-affinity complexes with their cognate target membrane proteins (t-SNARE), such as syntaxins and SNAP-25 proteins (synaptosome-associated protein of 25 kDa) (Cheatham et al., 1996). VAMP proteins associated with GLUT4 vesicles may serve as a v-SNARE (or SNAP receptor) that acts as a docking site for the GLUT4 vesicle forming complexes with t-SNAREs (such as syntaxin proteins) found in the plasma membrane (Thurmond, 2007). SNARE protein core complexes are also responsible for facilitating the downstream action of insulin on peripheral glucose disposal via the translocation to and integration of intracellular glucose transporter (GLUT4) vesicles into the plasma membrane of adipocytes and skeletal muscle (Sadoul et al., 1995; Wheeler et al., 1996).

**SNAP protein**

Soluble NSF-attachment proteins (SNAPs) are highly conserved proteins that participate in intracellular membrane fusion, vesicular trafficking, and the release of neurotransmitters and hormones through associations with N-Ethylmaleimide-Sensitive Fusion protein (NSF) and SNAP receptors (SNAREs) (Stenbeck, 1998). NSF plays a role in vesicular transport pathways. In mammals, there are three different isoforms of SNAPs, alpha, beta and gamma-SNAP. Only α- and γ-SNAP are widely expressed, with β-SNAP performing a brain-specific function (Woodman, 1997). SNAPs recruit NSF to the membrane after being bound to their membrane receptors (SNAREs). NSF, SNAPs and SNAREs form a heterooligomeric complex that is disrupted upon ATP hydrolysis by NSF, which is a prerequisite of membrane fusion. The NSF/SNAP proteins act after membrane fusion to disassemble the SNARE complex, thereby allowing the SNAREs to be reutilized for subsequent rounds of vesicle transport (Woodman, 1997).
**Insulin resistance**

A generally accepted view posits that insulin resistant condition in type-2 diabetes is caused by defects at one or several levels of the insulin-signalling cascade in skeletal muscle, adipose tissue and liver that quantitatively constitute the bulk of insulin-responsive tissues (Frojdo et al., 2009). Central insulin signalling is critical for the prevention of insulin resistance (Wilcox, 2005; Boura-Halfon and Zick, 2009; Mayer and Belsham, 2010). In the context of glucose homeostasis, insulin resistance is said to be present when the biological effects of insulin are less than expected for both glucose disposal in skeletal muscle and impaired inhibition of the production of hepatic glucose, and very low density lipoprotein, respectively resulting in hyperglycaemia and hypertriglyceridaemia (Abdul-Ghani and DeFronzo, 2010; Yki-Järvinen, 2010).

Skeletal muscles account for 30-40% of the body weight and it accounts for a large percentage of the whole body glucose uptake (DeFronzo et al., 1981b; Lee et al., 2000) and is an important site for insulin resistance (DeFronzo et al., 1985). In insulin resistant states, such as T2DM and obesity, insulin-stimulated glucose disposal in skeletal muscle is markedly impaired (DeFronzo et al., 1985; Leahy, 2005; DeFronzo, 2009; Abdul-Ghani and DeFronzo, 2010). The decreased insulin-stimulated glucose uptake is due to impaired insulin signalling and multiple post receptor intracellular defects including impaired glucose transport and glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis (Bajaj and DeFronzo, 2003; Bouzakri et al., 2005; Karlsson and Zierath, 2007).

Insulin-resistant states such as obesity are associated with reduced suppression of lipolysis in adipose tissue, which results in increased plasma FFA concentrations and ectopic fat deposition in liver and skeletal muscle, contributing to insulin resistance in these tissues (Donnelly et al., 2005; Frayn et al., 2006). Glucotoxicity and lipotoxicity as the consequences
of prolonged hyperglycaemia and hyperlipidaemia, can directly contribute to the development of insulin resistance and further deteriorate insulin sensitivity in skeletal muscle (Yki-Järvinen, 1992; Ostenson, 2001; Krook et al., 2004).

Insulin resistance is a common feature of several complex disorders comprising the dyslipidemia (increased plasma triglyceride/decreased HDL cholesterol), non-alcoholic fatty liver disease, normal ageing process (Defronzo, 1979), and in association with many disease states including heart failure (Swan et al., 1997), hypertension, chronic kidney failure (Bailey et al., 2006), polycystic ovary syndrome (PCOS) (Lankarani et al., 2009), myotonic dystrophy (Livingston and Moxley, 1994), and lipodystrophy (Simha and Garg, 2006).

**Management of type-2 diabetes mellitus**

Knowledge of the pathogenesis of T2DM is important in understanding the appropriate modes of treatment. T2DM is a heterogeneous syndrome due to the interaction of various environmental factors with multiple diabetogenic genes, which cause various combinations of beta cell failure and insulin resistance. Both defects are partly genetically and environmentally determined, and both are exacerbated by hyperglycemia (“glucose toxicity”) (Lillioja et al., 1993; Ferrannini, 1998). Weight reduction, exercise and dietary modification decrease insulin resistance and correct the hyperglycemia of T2DM in some patients (Barnard et al., 1994). Oral hypoglycemic agents and insulin therapy may be required to achieve satisfactory serum glucose levels. The goal in the management of type-2 diabetes with pharmacologic and nonpharmacologic therapies is important to maintain blood glucose concentrations within normal limits and includes patient education, evaluation for microvascular and macrovascular complications, treatment of glycemia, and minimization of cardiovascular and other long-term risks (Klein et al., 2004).
Non-pharmacological therapy

Diet is fundamental in the etiology and the management of T2DM (Krebs and Parry-Strong, 2013). Energy intake is a critical component of energy balance and body weight. Specific dietary components are implicated in diabetes pathogenesis and manipulation of these mooted as means to modify both weight and metabolic parameters (Krebs and Parry-Strong, 2013). People with diabetes need to adopt a healthful diet to achieve an effective treatment regimen (Franz et al., 1994). General guidelines include 50-60% of daily energy requirements derived from carbohydrates, low glycemic index foods, foods containing cereal fiber and a protein intake of at least 0.86 g/kg/day. The consumption of added sugars can be up to 10% of daily energy requirements. High consumption of vegetables, fruits, legumes, nuts, fish, cereals and oil leads to a high ratio of monounsaturated fatty acids to saturated fatty acids, a low intake of trans fatty acids, and high ingestion of dietary fiber, antioxidants, polyphenols (Barnard et al., 1994; Holt et al., 1995; Feskens and van Dam, 1999; Barnard et al., 2004; Barnard et al., 2005). Further, limited intake of total fat, especially saturated fats, with monounsaturated fatty acid (MUFA), appropriate use of nutritive and non-nutritive sweeteners, vitamins, minerals, well balanced diet and individualized physical activity are recommended for people with T2DM (Moran, 2004; Dedoussis et al., 2007).

Physical exercise is a key component of lifestyle modification that can help individuals prevent or control type-2 diabetes. Although diet is probably more important in the initial phases of weight loss, incorporating exercise as part of a weight-loss regimen helps maintain weight loss and prevent weight regain (Klein et al., 2004). Mild to moderate activity levels have been associated with a lower risk of developing diabetes or pre-diabetes (Wei et al., 1999). Greater levels of physical activity are associated with lower risks of developing diabetes in women compared with lesser levels of activity. These studies indicate that exercise
should be a mainstay of primary prevention of diabetes (Boulé et al., 2001; Bassuk and Manson, 2005).

Not only do these lifestyle modifications lower blood glucose concentrations, they also ameliorate many of the frequently co-existing risk factors for cardiovascular disease. Unfortunately, most patients are unable to achieve adequate control with lifestyle interventions alone, which should not detract from their critical role, since they enhance the effectiveness of medical regimens (Tiwari, 2015).

**Pharmacological therapy**

Diet and exercise are the first step in the treatment of T2DM. But if these measures alone fail to sufficiently control blood glucose levels, starting oral drug therapy is recommended. A number of oral anti-hyperglycemic agents have been introduced in recent years, each with its own mode of action. The choice of pharmacologic therapy should be based on a patient-centered approach with consideration of the efficacy, cost, potential side effects, comorbidities, hypoglycemia risk and patient preference.

Today's clinicians are presented with an extensive range of oral antidiabetic drugs for type-2 diabetes. The main classes are heterogeneous in their modes of action, safety profiles and tolerability. These main classes include agents that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reduce hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrate (alpha-glucosidase inhibitors) or improve insulin action (thiazolidinediones) (Kahn et al., 2014).

**Oral hypoglycemic agents**

**Biguanides**

Metformin is currently considered the first-line pharmacological therapy in type-2 diabetes mellitus (Consoli et al., 2004; Bennett et al., 2011), a recommendation that was
confirmed in the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) (Inzucchi et al., 2012). Metformin lowers blood glucose mainly by keeping the liver from releasing too much glucose by regulating gluconeogenesis and glycogenolysis and, to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues and may also increase glucose uptake in muscle via stimulation of AMP kinase (Stumvoll et al., 1995; Radziuk and Pye, 2001; Musi et al., 2002; Radziuk et al., 2003). In addition, it reduces the level of serum lipids, lipoproteins and HbA1c levels (Mehnert, 2001; Kirpichnikov et al., 2002). Metformin does not cause hypoglycemia and may lead to weight loss in some patients. Also, metformin may be successfully combined with all other glucose-lowering agents, including insulin (Bennett et al., 2011; Inzucchi et al., 2012). Its advantages are low cost and no weight gain. Most of the related side effects include metallic taste, diarrhea, bloating and cramping, gastrointestinal discomfort, nausea and Vitamin B12 deficiency has been reported with extended use (Luna and Feinglos, 2001; Wile and Toth, 2010; Davis, 2012) A rare, but more worrisome potential adverse effect is that of lactic acidosis with symptoms which include anorexia, nausea, hypernoea, abdominal pain and thirst (Alghabban, 2004; Misbin, 2004).

Thiazolidinediones

Thiazolidinediones (TZDs) increase insulin sensitivity by acting as PPARγ agonists (Nathan et al., 2009). TZD-induced activation of PPAR gamma alters the transcription of several genes involved in glucose and lipid metabolism and energy balance, including those that code for lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid binding protein, fatty acyl-CoA synthase, malic enzyme, glucokinase and the GLUT4 glucose transporter (Hauner, 2002). Thiazolidinediones also reduce circulating concentrations of pro-inflammatory cytokines that promote insulin resistance (eg, TNF-α and interleukin 6) and at
the same time increase concentration of adiponectin, which has insulin-sensitising and anti-inflammatory properties (Kendall et al., 2006).

Thiazolidinediones work by enhancing insulin sensitivity in both muscle and adipose tissue and to a lesser extent by inhibiting hepatic glucose production (Chakrabarti and Rajagopalan, 2002; Bays et al., 2004). It uniquely targets insulin resistance a core physiologic defect in those with T2DM – and by so doing significantly improve glucose control (Kendall et al., 2006; Colca et al., 2013). Pioglitazone is associated with bladder cancer have largely been allayed by subsequent evidence (Kuo et al., 2014; Tuccori et al., 2016). These agents tend to cause weight gain and peripheral edema and have been shown to increase the incidence of heart failure. They also increase the risk of bone fractures, predominately in women (Colhoun et al., 2012). The drawbacks of this drug includes fluid retention and adipose tissue accumulation (Ovalle and Ovalle-Berumen, 2002; Scheen, 2004).

**Sulphonylureas**

Sulfonylurea derivatives act by closing pancreatic cell potassium channels and subsequent opening of calcium channels (Rendell, 2004; Smith et al., 2010), which leads to enhanced insulin secretion and may slightly improve insulin resistance in peripheral target tissues (Luna and Feinglos, 2001). In general, they are well tolerated, with a low incidence of adverse effects, although there are some differences between the drugs in the incidence of hypoglycemia (Harrower, 2000). Sulfonylureas stimulate insulin secretion from pancreatic β-cells and are widely used to treat type-2 diabetes. Sulfonylureas work by binding to sulfonylurea receptor on beta cells of the pancreas thus enhancing insulin secretion from the pancreas blocking hepatic glucose production when being transported through the portal vein (Rendell, 2004). This class reduces glycosylated hemoglobin A1c (HbA1c) levels and fasting plasma glucose (FPG) concentrations (Turner et al., 1999; Hirst et al., 2013).
Hypoglycemia is the most prominent side effect of the sulfonylureas (Berger, 1984; Del Prato and Pulizzi, 2006).

**Meglitinides**

Meglitinides are secretagogue molecules with a more rapid anti-hyperglycemic action and a shorter duration than sulfonylureas, thus providing better control of post-prandial hyperglycemia and reducing the risk of late hypoglycemia (Landgraf, 2000; Dornhorst, 2001). The mechanism of action of meglitinides closely resembles that of the sulfonylureas coming under the class of insulin secretagogues (Bösenberg and van Zyl, 2008; Fisman et al., 2008) stimulating the release of insulin from the pancreatic beta cells. However, this action is mediated through a binding site on the “sulfonylurea receptor” of the beta cell, and the drug has somewhat different characteristics when compared with sulfonylureas (Fuhlendorff et al., 1998; Chakrabarti and Rajagopalan, 2002). Unlike the commonly used sulfonylureas, the meglitinides have a very short onset of action, high cost and a short half-life (Chiang et al., 2006; Smyth and Heron, 2006; Lorenzati et al., 2010).

**Alpha-glucosidase inhibitors**

Alpha-glucosidase inhibitors (AGIs) (Acarbose) are drugs that inhibit the absorption of carbohydrates from the gut and may be used in the treatment of patients with type-2 diabetes or impaired glucose tolerance (Van de Laar et al., 2006). AGIs delay carbohydrate digestion, prolongs the overall carbohydrate digestion time, and thus reduces the rate of glucose absorption (Godbout and Chiasson, 2007). After oral administration of AGI (acarbose), the postprandial rise in blood glucose is dose-dependently decreased, and glucose-induced insulin secretion is attenuated. Because of diminished postprandial hyperglycemia and hyper-insulinemia by acarbose, the triglyceride uptake into adipose tissue, hepatic lipogenesis, and triglyceride content are reduced. Therefore, acarbose treatment not only
flattens postprandial glycemia, but also ameliorates the metabolic state in general (Scheen, 2003; Van de Laar et al., 2005; Van de Laar, 2008). The most bothersome side effects observed with these agents are gastrointestinal, including abdominal discomfort, bloating, flatulence and diarrhea but are reversible with discontinuation (Toeller, 1991; Reuser and Wisselaar, 1994; Van de Laar et al., 2005).

**Insulin therapy**

Insulin therapy is an effective method for reducing blood glucose levels in patients with type-2 diabetes mellitus (T2DM), and most patients with T2DM eventually require insulin replacement to attain and preserve satisfactory glycemic control (Nyenwe et al., 2011). Treatment with insulin is indicated when patients have markedly elevated blood glucose levels, experience profound weight loss, hypoglycemia or have a tendency toward ketosis (Donnelly et al., 2007; Hawkes et al., 2014).

Many side effects of insulin therapy and other oral hypoglycemic agents necessitate the use of more effective and safer antidiabetic drugs. Despite the impressive advances in health sciences and medical care, there are many patients who are using alternative therapies alone or complementary to the prescribed medication because of their more effective, safer and less expensive management (Edwards, 2012).

**Significance of natural remedies**

During the past decade, the traditional systems have gained importance in the field of medicine. The World Health Organization estimates that 4 billion people, 80% of the world population in the developing countries, presently use herbal medicine for some aspect of primary health care (Orisatoki and Oguntibeju, 2010; Namuddu et al., 2011; Ekor, 2014). Many drugs commonly used today are of herbal origin because of their safety, quality, and efficacy (Yuan et al., 2016). Herbs had been used by all cultures throughout history. Herbal
medicine is a major component in all indigenous traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional, oriental and native American Indian medicine (Pan et al., 2014). Indeed, about 25% of the prescription drugs dispensed in the US contain at least one active ingredient derived from plant material (Verma and Singh, 2008; Aziz et al., 2014; Newman and Cragg, 2016). Therefore, it would be practical to treat various diseases with plant-derived compounds. Surveys from Europe and the United States have demonstrated a sharp rise in the use of botanical drugs within a few years and up to 65% of patients with various diseases take herbal preparations (Ghosh et al., 2011). Particularly, phenolics are considered as potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, cancer, diabetes, cardiovascular dysfunction, inflammatory diseases and aging (Srinivasan et al., 2007).

Herbal drugs are considered free from side effects than synthetic one. They are less toxic, relatively cheap and popular (Sen et al., 2010; Schaffer et al., 2016). In India, medicinal plants have been used as natural medicine since the days of Vedic glory. Many of these medicinal plants and herbs are part of our diet as spices, vegetables and fruits. There are several medicinal plants mentioned in the Indian system of medicine for the management of diabetes, which is effective given either alone or in combination (Rizvi and Mishra, 2013; Prakash et al., 2015).

**Management of T2DM with traditional medicine**

While there are biomedical drugs for managing different types of diabetes mellitus, patients with diabetes use other complimentary/traditional herbs as well because of its efficacy and lesser side effects. Herbs have been reported as one of the remedies used for treatment by patients with diabetes in various countries such as India, China, Vietnam and Oman (Jung et al., 2006; Modak et al., 2007; Hoa et al., 2009; Al-Kindi et al., 2011). The
efficacy of traditional herbs in the treatment of diabetes is still mixed. Studies have demonstrated that herbs can delay the progress of diabetic complications (Li et al., 2004; Feng et al., 2005; Jung et al., 2006). A list of medicinal plants with proven antidiabetic and related beneficial effects is compiled. These include, *Allium sativum*, *Eugenia ambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Cephalandra indica*, *Syzygium jambolanum*, *Trigonella foenum graecum* and *Withania somnifera* (Grover et al., 2002; Basha et al., 2012; Sujatha et al., 2012). One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions. Studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic complications (Rochette et al., 2014; Ayeleso et al., 2016).

*Allium sativum* (garlic) has been used for medicinal purposes around the world. In clinical trials, garlic supplementation among patients with dyslipidemia produced modest reduction in total cholesterol with no significant changes in LDL or HDL cholesterol levels (Stevinson et al., 2000; Reinhart et al., 2009). It also showed significant improvement in blood pressure levels in patients. Animal studies have suggested that the chemical components of garlic may increase insulin secretion or decrease degradation (Ajabnoor, 1990; Ried et al., 2008; Reinhart et al., 2009). *Aloe vera* plant has been used orally as a traditional treatment for diabetes. The gel derived from the meaty pulp of the leaf, may reduce blood glucose (Ghannam et al., 1986; Ajabnoor, 1990). Patients with type-2 diabetes who were given aloe gel juice reported decreases in fasting blood glucose during 6 weeks. *Coccinia indica* (ivy gourd) the creeper plant is prescribed in Ayurveda for the treatment of diabetes.
Coccinia may reduce blood glucose in a mechanism similar to insulin (Kamble et al., 1998). Clinical trials have suggested decrease in fasting blood glucose without adverse effects among type-2 diabetes patients after administration of Coccinia (Azad et al., 1979; Kuriyan et al., 2008). In one of the study 60 subjects were treated with Coccinia and the patients who received the herb had a 16% decrease in fasting blood glucose (Kuriyan et al., 2008). *Trigonella foenum graecum* (fenugreek) often flavors Indian food also used as a medicine in India and China. Experimental studies and clinical trials with type-1 and type-2 patients showed a potential effect on carbohydrate absorption and increased insulin secretion (Yeh et al., 2003; Srinivasan, 2005). *Momordica charantia* (bitter melon) vegetable is known as bitter melon, or “vegetable insulin.” Studies reported that it may reduce blood glucose in patients with diabetes (Krawinkel and Keding, 2006; Leung et al., 2009). Potential mechanisms of action for bitter melon are decreased hepatic glucose production, increased hepatic glycogen synthesis, and insulin-mimetic activity. *Gymnema sylvestre* (gymnema) has been used for two centuries in Ayurveda for the treatment of diabetes. This herb has demonstrated glucose lowering effects in animal and human studies, perhaps functioning as an insulin secretagogue (Shanmugasundaram et al., 1990a; Preuss et al., 1998). Gymnema has been studied as an adjuvant therapy to conventional care in patients with type-1 and type-2 diabetes, and it was reported to have significant improvement in fasting blood glucose and glycated hemoglobin levels among patients (Baskaran et al., 1990; Shanmugasundaram et al., 1990b). *Cinnamomum verum* (Cinnamon) one of the most widely used flavoring agents in the food and beverage industry worldwide has also been well recognized for its medicinal properties since antiquity (Jayaprakasha and Rao, 2011). Cinnamon is used as both a preventive and therapeutic supplement for many ailments including, type-2 diabetes, insulin resistance, metabolic syndrome, hyperlipidaemia and arthritis (Rafehi et al., 2012). Cinnamon is reported to have anti-diabetic properties, in addition to which, it is also perceived to have anti-oxidant,
anti-inflammatory and anti-bacterial properties (Brahmachari et al., 2009; Birdee and Yeh, 2010).

Plants possessing antidiabetic properties may be suitable as adjunct to the existing therapies or as a prospective source of new glucose lowering compounds. Herbal medicines are becoming immensely popular among the masses for being cost effective and with relatively few side effects (Thattet and Dahanukar, 1989; Prasad et al., 2009). Although plant based medicines have been used traditionally in treating diseases throughout the world, the mechanism of most of the herbs is still to be defined and standardized. More than 400 plant species having antihyperglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus (Jung et al., 2006; Campbell, 2009; Malviya et al., 2010; Patel et al., 2012b; Tiwari, 2015). These traditional approaches might offer a natural key to unlock diabetic complications.

**Ferulic acid**

Epidemiological studies have linked the consumption of whole grain products to the prevention of chronic diseases such as type-2 diabetes, cardiovascular disease and some type of cancers. Grain is an important source of numerous phytochemicals with potential biological activity, such as phenolic compounds (Liu et al., 1999; Anderson, 2004; Liu, 2007). Ferulic acid, (4-hydroxy-3-methoxycinnamic acid) is an extremely abundant and nearly ubiquitous phenolic cinnamic acid derivative with molecular formula C₁₀H₁₀O₄. In nature ferulic acid is biosynthetically comes from plants via cinnamic acid by the shikimic acid pathway (Graf, 1992; Rosazza et al., 1995).

Ferulic acid was chemically synthesized in 1925 by the amine-catalyzed condensation of vanillin with malonic acid (Dutt, 1925). In plants, ferulic acid is found in conjugated form. It
is widely linked to various carbohydrates as glycosidic conjugates, and it occurs as various esters and amides with a wide variety of natural products (Strack, 1990). Ferulic acid isolated from plants usually exists as the trans isomer. Since steryl ferulates were isolated from rice bran oil (Oryza sativa L.) and contained a hydroxyl group, it was conveniently named oryzanol (Kaneko and Tsuchiya, 1954). Ferulic acid and its derivatives are the members of a class of phenolic natural antioxidants, and many natural and presumed functions and potential uses of these compounds can be attributed to this property (Graf, 1992). Ferulic acid occurs in rice, wheat, oat, barley, olives, sorghum, tree bark, roasted coffee, forage, tomatoes, peas, vegetables, citrus fruits and leaves, asparagus, berries, and many other plants (Fausch et al., 1963; Fujimaki et al., 1977; Meyer et al., 1991; Bourne and Rice-Evans, 1998; Mattila et al., 2006; Mattila and Hellström, 2007; Kumar and Pruthi, 2014). Ferulic acid is found ubiquitously in the cell wall of woods, grasses, and corn hulls, generally localized in the bran fraction of seeds, wheat aleurone layers and wheat and barley scutella. Much of the ferulic acid occurs as esters in many plants. It is covalently conjugated with mono and disaccharides, plant cell wall polysaccharides, glycoproteins, lignin, beta cyanins, and other insoluble carbohydrate biopolymers of cell walls (Fulcher et al., 1972; Smart and O'Brien, 1979). Ferulic acid together with dihydroferulic acid, is a component of lignocelluloses, conferring cell wall rigidity by cross linking lignin and polysaccharides.

Experimental studies showed that the major and most potentially important site of ferulic acid absorption is the colon because ferulic acid is released from parent compounds or from the food matrix by microbial cinnamoyl esterase (Couteau et al., 2001). These cinnamoyl esterases are located mainly in the lumen of the large intestine, with only small amounts in the small intestine mucosa and possibly in pancreatic secretions (Andreasen et al., 2001). Approximately 95% of the total release of feruloyl groups occurs in human colon after release of feruloylated oligosaccharides by xylanases (Kroon et al., 1997). Moreover, ferulic
acid found in food esterified with carboxylic acids could also be released and absorbed in the colon (Herrmann, 1989). Na+-dependent, carrier-mediated transport process is involved in the uptake of cinnamic acid and ferulic acid across the brush border membrane of rat jejunum (Wolffram et al., 1995). It has been shown that ferulic acid might be transported across the intestinal epithelial cells by monocarboxylic acid transporters (MCTs) (Konishi and Shimizu, 2003).

Many different factors can influence the bioavailability of a compound. The first factor is the bioaccessibility or availability for absorption in the gastrointestinal tract (Stahl et al., 2002). Ferulic acid reported to have more bioavailability than other dietary flavonoids and phenolics. It stays in blood for longer time than other antioxidants such as vitamin C (Srinivasan et al., 2007). The binding of ferulic acid to polysaccharides may limit its bioavailability (Stahl et al., 2002).

**Biological significance of ferulic acid**

Studies have shown that ferulic acid exhibits a wide range of beneficial effects against various diseases in prevention and/or treatment including diabetes, cancer, hypertension, atherosclerosis and Alzheimer’s disease (Srinivasan et al., 2007; Zhao and Moghadasian, 2008). Antioxidant activity is one of the best documented biological activities of ferulic acid.

**Antioxidant effect of ferulic acid**

Transition metals, particularly iron, play a crucial role in oxygen radical reactions and subsequent oxidative damage to biological materials. These reactions often involve iron-catalyzed activation of oxygen to form superoxide anion radical, hydrogen peroxide, and highly reactive hydroxyl radical via the Fenton reaction. Formation of OH° strictly requires the availability of at least one free iron coordination site through which the redox shuttle can occur (Graf et al., 1984; Aust et al., 1985). Chelating agents have the ability to completely
block the catalytic activity of iron. In view of this, ferulic acid which has carboxyl and phenolic hydroxyl groups serves as a potential candidate for chelating iron.

Ferulic acid derives most of its antioxidant potential from its radical scavenging ability (Graf, 1992). It may partially act as an antioxidant by virtue of strongly mitigating the harmful effects of UV radiation. Ferulic acid possesses three distinctive structural motifs that can possibly contribute to the free radical scavenging capability of this compound. An electron donating groups present on the benzene ring (3 methoxy and more importantly 4-hydroxyl) give rise to terminate the free radical chain reactions (Graf, 1992). In addition to that carboxylic acid group in ferulic acid with an adjacent unsaturated C-C double bond gives additional attack sites for free radicals and thus prevent them from membrane damage. The carboxylic acid group also acts as an anchor of ferulic acid, by which it binds to the lipid bilayer, providing some protection against lipid peroxidation (Graf, 1992; Srinivasan et al., 2007; Zhao and Moghadasian, 2008).

**Antidiabetic effects of ferulic acid**

In hyperglycemia, auto-oxidation of glucose increases the formation of free radicals beyond the capacity of body’s defense system to neutralize it thereby causing oxidative stress, the major cause and consequence of diabetes mellitus (Giacco and Brownlee, 2010). Administration of ferulic acid reduced the blood glucose level in streptozotocin-induced diabetic animals. Since it has a potential antioxidant activity, it helps to neutralize the free radicals produced by streptozotocin in the pancreas and thereby decrease the toxicity of streptozotocin. This decreased oxidative stress/toxicity on the pancreas may help the beta cells to proliferate and secrete more insulin. This increased insulin secretion can cause increased utilization of glucose by the extra hepatic tissues and thereby decrease the blood glucose level (Balasubashini et al., 2004). Oral administration of ferulic acid at low dosage (0.1% or 0.01%)
suppressed the hyperglycemia associated with STZ-induced diabetes in mouse model and 0.05% ferulic acid suppressed the blood glucose level effectively in KK-Ay mice, a type-2 diabetic model (Ohnishi et al., 2004).

Treatment with various amide compounds of ferulic acid stimulated the insulin secretion in RIN-5F cells suggesting the insulin releasing property of ferulic acid (Nomura et al., 2003). The presence of m-hydroxy or p-methoxy residues in cinnamic acid is important substituent for insulin secreting activity of ferulic acid in perfused rat pancreas and pancreatic β-cells (INS-1) which is associated with an increase in intracellular calcium (Adisakwattana et al., 2008; Yibchok-anun et al., 2008). Balasubashini et al. (2004) reported that 10 & 40mg of ferulic acid decreased the blood glucose in female Wistar rats with STZ-induced diabetes. Jung et al. (2007) demonstrated using diabetic mice, that ferulic acid increases the activity of glucokinase, a key enzyme in the regulation of blood glucose levels. Cinnamic acid derivatives have the inhibitory activity against rat intestinal α-glucosidase and porcine pancreatic α-amylase in vitro. Those derivatives are used to find effective inhibitors from natural sources that could be used in prevention and treatment of diabetes mellitus (Adisakwattana et al., 2009). Ferulic acid treatment regulates the blood glucose level by elevating glucokinase activity and synthesis of glycogen in the liver (Jung et al., 2007). Son et al. (2011) demonstrated that treatment with oryzanol from rice bran and ferulic acid could reduce the risk of high-fat diet-induced hyperglycemia via regulation of insulin secretion and hepatic glucose-regulating enzyme activities. Treatment with 50mg of ferulic acid acts as a protective agent by altering oxidative stress, expression of pro-inflammatory cytokines and apoptosis in diabetic rats (Roy et al., 2013). Ferulic acid is found to have a positive effect on high fat diet-induced deleterious effects on the liver of type-2 diabetic rats by improving insulin sensitivity and hepatic glycogenesis but inhibits gluconeogenesis, reduces the GLUT2 expression by impairing the interaction between transcription factors (SREBP1c, HNF1α and HNF3β) and GLUT2 gene promoter and also decreases the negative regulators of insulin signalling to maintain normal glucose homeostasis (Narasimhan et al., 2015a, b).