1. INTRODUCTION

1.1 Diabetes

Diabetes mellitus (DM) is a degenerative progressive disease characterized by insulin deficiency which is characterized as hyperglycemia which occurs due to insulin resistance. The fasting as well as post-prandial blood glucose level gets elevated, thus exposes the patient to acute and chronic complications which leads to blindness, kidney failure, heart disease, stroke and amputations and many more other complications. Improving the glycemic control helps to lower the risk of these complications. Owing to the nature of this progressive disease, an ever evolving treatment strategy is very much necessary to be planned to maintain glycemic control. Diabetes is a progressive degenerative metabolic disorder resulting from an insulin secretion defect, insulin action, or both. Insulin deficiency in turn thus leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism pathway. It is the most common endocrine disorder and by the year 2016, it is estimated that more than 300 million people worldwide will have DM and 400 million will subsequently have the disease by 2025. As this disease progresses tissue and vascular damage leads to very severe diabetic complications like retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a broad range of heterogenous disease. The threshold for fasting glucose was altered from 7.7 Mol/L (140 mg/dl) to 7.1 mmol/L (127 mg/dl); however the 2-h glucose criterion remains as = 11.2 Mol/L (200 mg/dl). Diabetes mellitus have reached global pandemic proportions with India being named as ‘diabetes capital’.  

1.2 Classification of Diabetes-

1. Type1 (β-cell destruction, usually leading to complete insulin deficiency; Immune-mediated diabetes)

This type of diabetes, which usually accounts for only 5–10% of those with diabetes, previously, encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes.
Causes-
- Genetic predisposition.
- Environmental exposure to toxin, stress and virus.
- Autoimmune reaction: beta-cells that produce insulin in the pancreas are completely destroyed. When 85-90% of the beta-cells are completely destroyed, these symptoms occur.

Characteristics-
- Usually occurs before 30 years of age, but it can occur at any phase of life. Peak time is during puberty, around 10-12 years of age in girls and 12-14 years in boys.
- Abrupt onset of signs and symptoms of hyperglycemia are increased thirst and hunger, frequent urination, weight loss, and fatigue.
- It is Ketosis prone.

Treatment-
- Insulin intake by injection with syringes or pumps.
- Maintaining proper diet.
- Regular Exercise.

2. Type 2 (ranges from predominantly insulin resistance with insulin deficiency to insulin secretory defect with insulin resistance)\(^{3,4}\)

This type of diabetes, which holds for~91–95% of population with diabetes, also called as non-insulin-dependent diabetes, type II diabetes, or adult-onset diabetes.

Causes-
- Human body is unable to utilize insulin that the body produces because of cell-receptor defect; glucose is unable to be absorbed into cells for fuel as result of Insulin resistance.
- Low insulin secretion: pancreas does not secrete sufficient insulin in response to glucose levels.
- Excess production of glucose from the liver: As a result of defective insulin secretory response; dawn phenomenon is an example.

Characteristics-
- Occurs after 30 years of age, but is now a day’s occurring in children and adolescents.
Increased prevalence in some ethnic groups, e.g., African Americans, Hispanic/Latino, Native Americans, Asian Americans, and Pacific Islanders.

- Strong genetic predisposition.
- Frequently obese.
- Not prone to ketoacidosis until late in course or with prolonged hyperglycemia.
- May or may not have symptoms of hyperglycemia.
- May also have extreme tiredness, blurred vision, delayed healing, numbness and tingling of hands and feet, recurring yeast infection.
- Children between the ages of 10-19 that have one or more of the following are at an increased risk.
- Family history.
- Member of certain ethnic populations.
- Overweight.
- Sedentary lifestyle.
- Pre-puberty.
- Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans [dirty-neck syndrome], hypertension [high blood pressure], dyslipidemia [lipoproteins imbalances], and polycystic ovarian syndrome [PCOS]).

Treatment-
- Diet/weight management.
- Exercise/increase physical activity.
- Oral hypoglycemic/anti hyperglycemic agents, insulin sensitizers, or insulin.
- Treatment of comorbid conditions (e.g., hypertension, lipid abnormalities).  

3. **Gestational Diabetes Mellitus (GDM)** 3,4

Increased levels of blood sugar during pregnancy are usually called as gestational diabetes.

**Causes**-
- Insulin resistance which occurs due to pregnancy.
- Genetic predisposition.

**Characteristics**-
- Carbohydrate intolerance during pregnancy is identified during 1-hour screen using a 50-g oral glucose load (which is performed between 24th and 28th week of gestation...
unless otherwise it is indicated). If the 1-hour screen for glucose is >140 mg/dl (>7.8 mol/l), a full diagnostic 100-g, 3-hour oral glucose tolerance test (OGTT) is indicated.

Treatment:

✓ Diet: provide sufficient calories without hyperglycemia or ketonemia.
✓ Daily workout: program that does not cause fetal distress, contractions, or hypertension (>140/90 mmHg).
✓ Insulin: if unable to maintain consistent blood glucose <95 mg/dl fasting (<5.3 mmol/l) and <140 mg/dl (<7.8 mmol/l) 1 hour postprandial and <120 mg/dl (<6.7 mol/l) 2 hours postprandial.

4. Other specific types

✓ Genetic defects of β-cell function in the body.
✓ Genetic defects in insulin secretion in the body.
✓ Diseases of the exocrine pancreas in body.
✓ chemical induced or drug-induced cytomegalovirus and others
✓ Unique forms of immune mediated diabetes also occurs
✓ Other genetic syndromes sometimes are also associated with diabetes

1.3 Cellular Mechanisms of Insulin Resistance

Mechanism of fatty acid–induced insulin resistance in skeletal muscle as of proposed by Randle et al.; an elevated level in fatty acid concentration results in an increased level of the intra mitochondrial acetyl-coA/CoA to that of NADH/NAD⁺ ratios with subsequent inactivation of pyruvate dehydrogenase.

This in turn causes citrate concentrations to rise subsequently, which leads to inhibition of phosphofructokinase. Subsequent elevated level in intracellular glucose-6-phosphate concentration would inhibit hexokinase II activity which would result in arise in intracellular glucose concentration and subsequent decrease in muscle glucose uptake.

Suggested alternative mechanism for fatty acid–induced insulin resistance in human skeletal muscle; rise in delivery of fatty acids to muscle or a decrease in intracellular metabolism of fatty acids leads to an elevation in intracellular fatty acid metabolites such as diacyl glycerol, fatty acyl CoA and ceramides.
These key metabolites activates a serine/threonine kinase cascade leading to phosphorylation of serine/threonine binding sites on insulin receptor substrates (IRS-1 and IRS-2), which in turn retards the ability of the insulin receptor substrates to activate PI 3-kinase. As a result, glucose transport activity and other events downstream of insulin receptor signaling are diminished.\textsuperscript{5}

HK, hexokinase II; PFK, phosphofructokinase; PDH, pyruvate dehydrogenase; PKCq, protein kinase

2. GLYCOGEN SYNTHASE KINASE-3 INHIBITORS:

Glycogen synthase kinase (GSK-3) is a serine/threonine kinase that phosphorylated glycogen synthase and inhibits its activity. Thus, inhibition of GSK3 is expected to activate glycogen synthase and promote glucose uptake into muscle.

Over the past few years there has been much interest within the pharmaceutical industry in identifying compounds that inhibit GSK3 as possible insulin mimetic/sensitizing drugs. This interest has been heightened by the report that the level and activity of GSK3 is moderately elevated in diabetic and obese strains of mice. In liver cells, these compounds mimic insulin signaling, as expected by promoting the dephosphorylation and activation of glycogen synthase, thereby facilitating the conversion of extracellular glucose into glycogen. However, interestingly, they also mimic a further action of insulin, namely its ability to repress the expression of the genes encoding glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. The enzymes that control hepatic gluconeogenesis. These recent observations are exciting, since they imply that GSK3 inhibitors may suppress hepatic glucose output, as well as aiding glucose disposal by the tissues. Such drugs may therefore have greater therapeutic potential for the treatment of type II diabetes than recognized previously. Among the reported GSK-3 inhibitors, CHIR98014, \textsuperscript{37} and CHIR98023, \textsuperscript{38} increased glucose uptake in human skeletal muscle cell culture.\textsuperscript{8,13}
2.1 Glycogen Synthase Kinase-3 Enzyme

GSK3 was discovered over 20 years ago as one of several protein kinases that phosphorylated and inactivated glycogen synthase, the final enzyme in glycogen biosynthesis. Three isoforms of GSK-3 have been identified in mammalian cells, GSK-3α, GSK-3β, and GSK-3β2 (a splicing variant of GSK-3β). The α and β isoforms show a substantial deviation in protein sequence, mostly outside the kinase core (308 residues), but the core has 97% sequence similarity and overall 91% identity.16, 17.

Glycogen Synthase Kinase-3β (Gsk-3β) Structure:

GSK-3β is comprised of 12 exons in humans and 11 exons in mice with the ATG start codon located within exon 1 and the TAG stop codon found in the terminal exon. The gene product is a 46 kDa protein consisting of 433 amino acids in the human and 420 amino acids in the mouse. Figure 1 shows the overall structure of GSK-3β. It is similar to the Ser/Thr kinases.33, 34 The N-terminal domain is comprised of the first 135 residues and forms a 7-strand β-barrel motif. A small linker region connects the N-terminal domain to the central α-helical domain formed by residues 139 through 342. The ATP-binding site lies at the interface of the N-terminal and α-helical domains. Residues 343 through 433 form the C-terminal domain, which is outside of the classical Ser/Thr kinase core fold. These residues form a helix/loop domain that interacts with the core α-helical domain. The N-terminal amino acids 78 through 92 are necessary for association with p53 (Figure 1). The activity of GSK-3β can be reduced by phosphorylation at Ser-9. Several kinases are able to mediate this modification, including gp 70S6 kinase, p90RSK, PKC, and Akt. In opposition to the inhibitory phosphorylation of GSK-3β at Ser-9, phosphorylation of GSK-3β at Tyr-216 by ZAK1 or Fyn increases its
enzyme activity\textsuperscript{16, 17}

Fig no 1.1 Glycogen synthase kinase-3\(\beta\) (GSK-3\(\beta\)) structure.

GSK-3\(\beta\) is a 433 residue protein consisting of 3 distinct structural domains. The N-terminal domain (yellow) consists of the first 134 residues and forms a 7-strand \(\beta\)-barrel. A short linker from the N-terminal domain, residues 135–151, connects the N-terminal domain to the \(\alpha\)-helical domain (magenta). The \(\alpha\)-helical domain is composed of residues 152–342. Sandwiched between the N-terminal and \(\alpha\)-helical domain is the ATP-binding site. The C-terminal domain consists of residues 343–433 (blue). A strand diagram of GSK-3\(\beta\). Phosphorylation of Ser-9 inactivates the enzyme, while phosphorylation of Tyr-216 activates. The p53 association region and basic domain region are both located in the N-terminal domain.

2.2 Regulation of GSK-3 by Insulin and Growth Factors

The binding of insulin to its receptor in liver, adipose tissue and muscle, triggers the phosphorylation of IRS proteins and their recruitment to the plasma membrane. The IRS proteins in turn become tyrosine-phosphorylated, recruiting PI 3-kinase (PI3K) to the membrane, where it produces the second messenger Ptd Ins (3, 4,5) \(P_3\) (PIP3). This molecule binds to PDK1 and PKB co-localizing them at the plasma membrane and allowing the former to activate the latter. Active PKB then inhibits GSK3 by phosphorylating Ser21 (GSK3\(\alpha\)) and Ser9 (GSK3\(\beta\)). As a result, the residues on glycogen synthase and the e-subunit of eukaryotic initiation factor 2B (eIF2B, lilac) that are targeted by GSK3 undergo a partial dephosphorylation, thereby increasing their activity and hence stimulating glycogen and protein synthesis.\textsuperscript{13}
Normally insulin induced inactivation of GSK-3 contributes to glucose uptake and glycogen synthesis. Molecular mechanism of insulin signaling via insulin receptor substrate (IRS). IRS-1 is the immediate substrate of insulin receptor tyrosine kinase, which phosphorylates the protein on multiple tyrosine residues in response to insulin. In addition, IRS-1 is predominantly phosphorylated on serine/threonine residues in the absence of stimuli. It appears that this type of phosphorylation of IRS-1 converts the protein from a positive to a negative regulator of insulin receptor signaling. Yet its serine/threonine phosphorylation (pS) results in the opposite effect, presumably by direct interaction of IRS-1 with the insulin receptor. These studies implicated serine/threonine protein kinases as important regulators in insulin resistance. Phosphorylation of IRS-1 on multiple serine residues by GSK-3 impaired insulin receptor tyrosine kinase activity and insulin action in intact cells. This notion fits well with the fact that GSK-3 is constitutively active and phosphorylates IRS-1 in the absence of stimulus. Thus, GSK-3

Figure 1.2: Insulin stimulates glycogen synthesis via the inhibition of GSK3
serves as a ‘gatekeeper’ to limit activation of insulin receptor signaling. In the absence of insulin, GSK-3 maintains the phosphorylation state of the multiple serine residues on IRS-1, thereby limiting insulin receptor signaling. In the presence of insulin, GSK-3 is inhibited, and tyrosine phosphorylation of IRS-1 mediates the downstream insulin signaling pathway. Thus it is clear that GSK-3 inhibits insulin receptor coupled protein IRS-1, which in turn inhibits glycogen synthesis and glucose uptake.\textsuperscript{10}

**Figure 1.3:** Insulin receptor substrate 1 (IRS-1) phosphorylation regulates insulin signaling

Most kinase inhibitors act by competition with either ATP or metal-binding sites that are involved directly in the catalytic process. However, small-molecular-weight compounds might regulate GSK-3 activity by inhibiting the protein–protein interactions that are necessary for binding of substrate by modulating the Tyr216 (GSK-3β) and Tyr279 (GSK-3α) activation sites and the Ser9 (GSK-3β) and Ser21 (GSK-3α) inhibition sites, and by interfering with the intracellular targeting domain of GSK-3. Inhibition of the interaction between the docking protein and the priming kinase might change the substrate specificity of GSK-3.\textsuperscript{20}

**2.3 Therapeutic Potential of GSK-3 Inhibitors for the Treatment of Diabetes**

Type II or non-insulin-dependent diabetes mellitus (NIDDM) accounts for approx. 90% of all cases of the disease. The initial stages are characterized by insulin resistance, i.e. an
inability of the peripheral tissues (muscle, fat and liver) to respond correctly to the insulin that is secreted from the pancreas.\textsuperscript{7-9}

However, as the disease progresses, the pancreas are no longer able to produce enough insulin to counteract the resistance and daily injections of exogenous insulin then become necessary. Thus drugs are urgently needed to combat resistance, allowing the tissues to respond much better to the insulin already present. Over the past few years there has been much interest within the pharmaceutical industry in identifying compounds that inhibit GSK3 as possible insulin mimetic sensitizing drugs.\textsuperscript{7-9}

This interest has been heightened by the report that the level and activity of GSK3 is moderately elevated in diabetic and obese strains of mice. Investigators at GlaxoSmithKline have recently developed a class of maleimides that are potent and relatively selective inhibitors of GSK3. In liver cells, these compounds mimic insulin signaling, as expected by promoting the dephosphorylation and activation of glycogen synthase, thereby facilitating the conversion of extracellular glucose into glycogen. However, interestingly, they also mimic a further action of insulin, namely its ability to repress the expression of the genes encoding glucose-6phosphatase and phosphoenolpyruvate carboxykinase, the enzymes that control hepatic gluconeogenesis. These recent observations are exciting, since they imply that GSK3 inhibitors may suppress hepatic glucose output, as well as aiding glucose disposal by the tissues.\textsuperscript{7-9}

Such drugs may therefore have greater therapeutic potential for the treatment of type II diabetes than recognized previously

2.4 Current Status of GSK-3 Inhibitors

Lithium salts (Li+) weakly inhibit GSK-3 through competition with the binding of Mg\textsuperscript{2+}, the essential metal ion cofactor of the enzyme. Inhibition of GSK-3 by lithium salts causes enhanced glycogen synthase activity and reduced phosphorylation of various GSK-3 substrates.

Based on the mechanism phosphorylation at Ser-9/Ser-21 several phosphopeptides, derived from the amino-terminal end of GSK-3\textbeta, have been produced in an effort to compete with the binding of substrates to the phosphate interaction site of the enzyme. One such phosphopeptide, Thr–Thr–pSer–Phe–Ala–Glu–Ser–Cys, was found to inhibit the phosphorylation of glycogen synthase.\textsuperscript{25}
High throughput screening of the SmithKline Beecham compound collection has identified 3-anilino-4-arylmaleimide (11) as potent GSK-3 inhibitor. Pharmacological studies conducted on maleimide derivatives, SB-216763 (12) and SB-415286 (13), have shown that they stimulated glycogen synthesis in human liver cells. SB-517955 (14) had the capacity to lower glucose level in animal models\textsuperscript{10}.

Johnson and Johnson Pharmaceutical Research and Development developed a novel series of macro cyclic bisindolyl maleimides containing linkers with multiple heteroatom’s’ having high selectivity for GSK-3\(\beta\). Another series developed by Johnson and Johnson is polyoxygenated bis-7-azaindolyl maleimides\textsuperscript{10}.

Glaxo SmithKline Research and Development afforded a series of pyrazolo [3,4-b] pyridines (15) and pyrazolo [3,4-b] pyridazines (16) with IC\textsubscript{50} value in nM range. Several compounds synthesized in this series may be useful in the treatment of diabetes mellitus.

Novo Nordisk reported the discovery of GSK-3 inhibitory activity within various chemical series, including substituted oxadiazepines. 1-(4-amino-1,2,5-oxadiazolyl)-1,2,3-triazole derivatives (17) and 2,4-diaminothiazoles (18, 19).\textsuperscript{1} Vertex Pharmaceuticals described the preparation of 4-arylpyrimidine-2-amines (20) and 4,5-dihydro-1H-pyrazole-5-one (21) as GSK-3 inhibitors. Their potential in treatment of type 2 diabetes is still to be evaluated\textsuperscript{10}.

Chiron Corporation claimed the discovery of several GSK-3 inhibitors comprising substituted 2-aminopyridazines (22), 2-aminopyrimidinesWO 02/020495 and 2-aminopyridines WO 99/065897. CT-98023 (23) developed by Chiron has shown good oral bioavailability, which reduced plasma glucose levels in fasted hyperglycemic rats, improved hyperglycemia and glucose disposal in diabetic mice\textsuperscript{10}.