Chapter 1

Review of Literature

1.1 Introduction

Lifestyle diseases are on the upswing with rising incidences of atherosclerosis, cardiovascular diseases, stroke, hypertension, obesity and diabetes mellitus (DM). DM is a severe and chronic condition that alters carbohydrate, protein and fat metabolism. It is manifested when the body cannot produce enough insulin, or cannot use the produced insulin effectively (Pramanik *et al*., 2018). With the rising global pervasiveness of DM, it has established itself as a chronic disease that permeates all ethnicities and economies, whilst gaining recognition as a public health priority in most countries (Unnikrishnan *et al*., 2016). According to recent statistics, 1 in every 11 adults (20-79 years), i.e., 463 million people have DM while 232 million remain undiagnosed, with 10% of global health expenditure allotted for DM (Saeed *et al*., 2019). The prevalence of DM worldwide is shown in Figure 1.1.

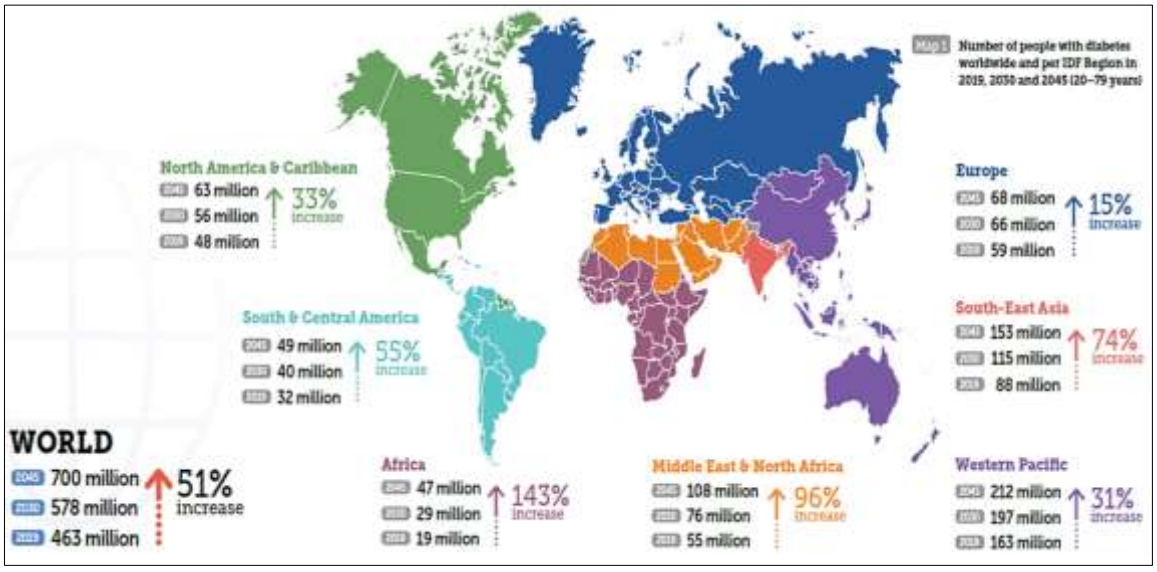

Figure 1.1. Worldwide prevalence of diabetes mellitus (IDF Diabetes Atlas, 2019)

1.1.1 The mechanism for regulation of blood glucose

Insulin is a 51 amino acid long peptide hormone, synthesised from the precursor polypeptide pre-proinsulin, which consists of 110 amino acids and is secreted by the β-cells of the islets of Langerhans of the pancreas. It is an anabolic hormone with mitogenic effects, responsible for maintaining normal blood glucose levels. Glucose is the principal stimulus for the synthesis and secretion of insulin, wherein glucose levels more than 3.9mmol/L stimulates insulin synthesis (Sprague and Arbelaez, 2011). Insulin promotes glucose uptake, glycogenesis, lipogenesis, and protein synthesis in skeletal muscle and fat tissue through the tyrosine kinase receptor pathway. In addition, insulin is the most important factor in the regulation of plasma glucose homeostasis, as it counteracts glucagon and other catabolic hormones such as epinephrine, glucocorticoid, and growth hormone.

Carbohydrates consumed postprandially, get hydrolysed into glucose in the intestine, which is then absorbed and transported through the blood to the entire body. As the blood glucose level increases, it signals the pancreatic β-cells to initiate insulin release into the bloodstream through glucose-stimulated insulin secretion (GSIS), as shown in Figure 1.2. The secreted insulin then triggers glucose uptake from the bloodstream by the principal insulin sensitive or insulin-responsive tissues, viz. skeletal muscle, adipose tissue and liver (Pramanik *et al*., 2018). When the insulin-stimulated glucose uptake by individual organs and their contribution to the whole-body glucose utilisation was analysed, the liver was found to be the major consumer of glucose. It plays a vital role in storing energy as glycogen and triglycerides. The rest of the glucose is utilized by skeletal muscle, adipose tissue and brain. Liver removes the monosaccharides and two-third of the glucose from circulation. It has a vast glycogen reserve, and its metabolic activities are essential for providing fuel to the brain, muscle, and other peripheral organs. For the brain, glucose is virtually the sole fuel, except during prolonged starvation, and since it does not store any glucose, it needs a steady and constant supply of glucose. Glucose is also the major fuel for skeletal muscle, in addition to fatty acids, and ketone bodies. About three-fourth of the total glycogen in the body is stored in the muscle and gets utilised during all activities (Berge *et al*., 2002). However, in DM, the body is constantly under a sense of fasting, wherein the pancreas produces less insulin due to β-cell loss or due to reduced GSIS or the insulin secreted cannot induce glucose uptake. Thus, the vigilant control over blood glucose is lost. The underlying reasons for this lack of glycaemic control are different in different forms of DM.

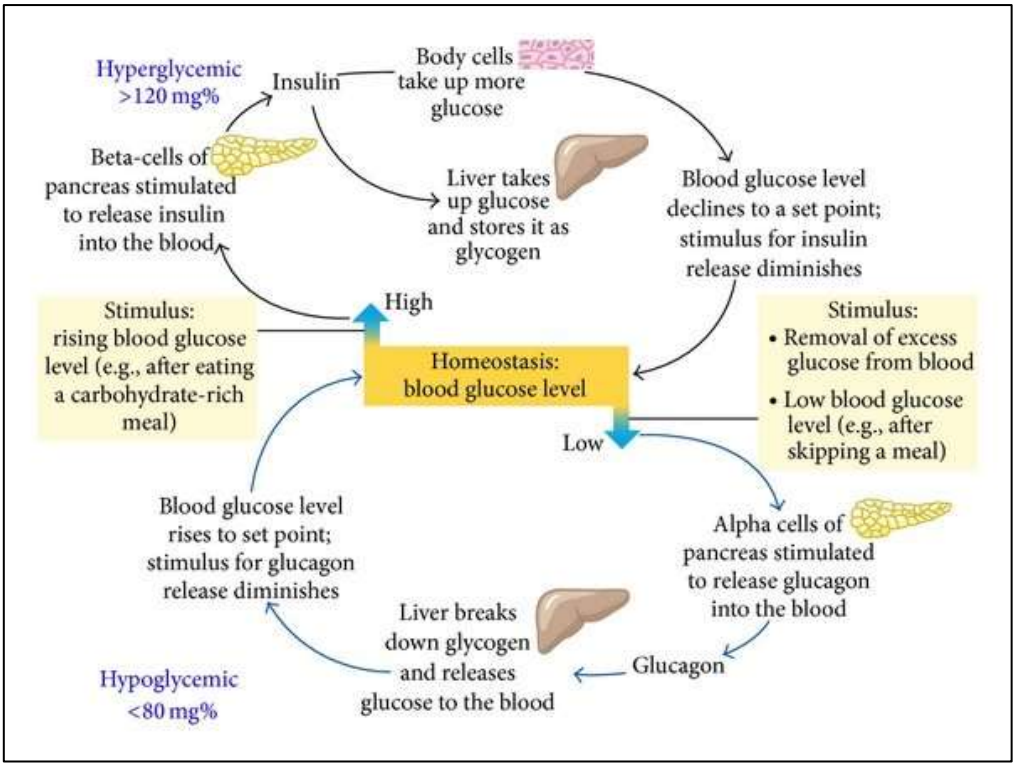

Figure 1.2: Regulation of blood glucose levels (Lowe, 2005).

1.1.2 Classification of diabetes mellitus

Diabetes mellitus is broadly classified into three types:

a. **Type 1 diabetes (T1D),** also known as insulin-dependent DM or juvenile onset DM.
b. **Type 2 diabetes (T2D),** also known as non-insulin dependent DM.
c. **Gestational diabetes (GD):** is a form of high blood sugar affecting pregnant women. Mothers developing gestational diabetes are predisposed to T2D later in their life.

1.1.3 Type 1 diabetes

In India, 77 million people suffer from T1D, which is expected to rise to 134 million by 2045, taking its rank to second most affected, globally (Saeed *et al*., 2019). T1D is also known as "juvenile/childhood-onset diabetes" or "insulin-dependent diabetes" as it is the primary cause of diabetes in children. It results from immune-mediated loss of endocrine pancreatic β-cells and is characterised by insulin deficiency (Redondo *et al*., 2018). The markers for T1D are low level of C-peptide (Jones and Hattersley, 2013), presence of autoantibodies against glutamic acid decarboxylase (GAD65) (Chiang *et al*., 2013), islet cells (ICA), islet antigen-2 (IA-2A) and zinc transporter (ZnT8) (Kawasaki, 2012). Nevertheless, people with T1D can live healthy lives with the provision of an uninterrupted supply of insulin.

1.1.4 Type 2 diabetes

T2D is the most common type of diabetes, accounting for around 90% of all diabetes cases (Saeed *et al*., 2019). Epidemiological data show alarming values that predict a worrisome projected future for T2D. According to the international diabetes federation (IDF), in 2019, diabetes caused 4.2 million deaths; and 463 million adults aged between 20 and 79 were living with diabetes, a number that is likely to rise up to 700 million by 2045. The causes of T2D are varied and poorly understood. It is a polygenic and multifactorial disorder strongly linked with overweight and obesity, increasing age and ethnicity, family history, along with cellular stress and mitochondrial dysfunction (Pramanik *et al*., 2018; Javeed *et al*., 2018; Pinti *et al*., 2019). T2D often remains asymptomatic for a long duration (prediabetes), resulting in an extended pre-diagnostic period. Hence, one-third to one-half of population with T2D may remain undiagnosed at any given point of time. Alarmingly, T2D has also become a concern in children and young people due to an increasing prevalence of obesity.

1.1.5 Gestational diabetes

Hyperglycemia in pregnancy (HIP) can be classified into two types, gestational diabetes (GD) or diabetes in pregnancy (DIP) (WHO guidelines, 2014; Hod *et al*., 2015). To be classified as GD, hyperglycemia should be diagnosed for the first-time during pregnancy (mainly after 24 weeks) (Hod *et al*., 2015). In contrast, DIP applies to those who have a history of diabetic hyperglycemia first diagnosed during pregnancy. Also, DIP may occur at any time during pregnancy, including the first trimester (Immanuel and Simmons, 2017). About 75–90% cases of HIP are GD (Guariguata *et al*., 2014). GD typically exists as a transient condition during pregnancy and resolves post-pregnancy. However, the relative risk of developing T2D increases with a history of GD.

1.2 Diagnosis

T1D can be diagnosed by the presence of symptoms viz. polyuria, polydipsia unexplained weight loss, and a random venous plasma glucose concentration ≥ 11.1 mmol/l or a fasting plasma glucose concentration ≥ 7.0 mmol/l (whole blood ≥ 6.1 mmol/l or HbA1c ≥ 6.5%). Prediabetes/non-diabetic hyperglycemia or intermediate hyperglycemia is marked by impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). IGT and IFG are vital because they signify a risk of the future development of T2D (Heianza *et al*., 2011; Tabák *et al*., 2012; Richter *et al*., 2018). As per WHO and IDF recommendations to detect IGT and

IFG, a two-hour oral glucose tolerance test (OGTT) must be performed. The diagnostic criteria for diabetes, IGT and IFG are summarised in Figure 1.3.

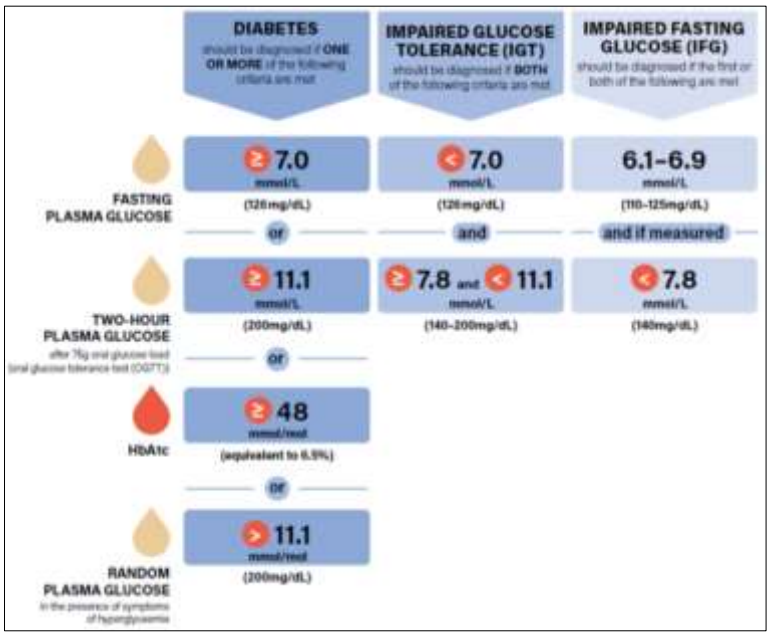

Figure 1.3. Diagnostic criteria for diabetes mellitus. (IDF, 2019)

1.3 Pathophysiology of DM

Loss of pancreatic β-cells and their function is the hallmark of DM. However, the pathogenesis behind it differs in T1D and T2D, while autoimmune attack leads to β-cell death in T1D (Ramos-Rodriguez *et al*., 2019), glucolipotoxicity leads to β-cell dysfunction in T2D.

1.3.1 Pathophysiology of type 1 diabetes

T1D is usually prevalent among individuals without a family history. Only 10–15% of T1D patients have a first or second-degree relative with the disease. Environmental factors also play an important role in the pathogenesis of T1D. Though the precise effect of these factors remains unclear, viruses (rubella, coxsackievirus B or enteroviruses), toxins and nutrients (cow's milk, cereals) have been noted to play a role. Classically, T1D is known to arise from the autoimmune destruction of β-cells of the endocrine pancreas. However, a small percentage of affected patients (<10%), with no evidence of autoimmunity, are classified as type 1B, and the pathogenesis in such cases are considered as idiopathic (Atkinson and Maclaren, 1994; Paschou *et al*., 2014). The human immune system distinguishes foreign from self and in this process, faces a huge variety of antigens. During T lymphocyte growth in the thymus and B lymphocyte in the bone marrow, potentially dangerous immune cells are negatively selected and eliminated (central tolerance). Self-reactive lymphocytes, which escape central tolerance mechanisms and end up in the periphery, naturally enter into

processes that either neutralise or suppress them (peripheral tolerance). Disorders of these immune mechanisms can result in various autoimmune conditions (Jnaeway *et al*., 2005). In recent years, it has been demonstrated that the subpopulation of T regulatory lymphocytes (Tregs) plays an important role in the immune response, especially for peripheral tolerance. It has been found that patients with T1D present quantitative and qualitative deficits in Tregs, which may explain the overshooting immune response, which eventually leads to the autoimmune response (Paschou *et al*., 2008; Paschou *et al*., 2010; Paschou *et al*., 2014). The destruction of β-cells in T1D occurs most probably via apoptosis. However, necrosis and necroptosis may also be important in humans (Eizirik *et al*., 2009; Cunha *et al*., 2012; Grieco *et al*., 2017). To date, several hypotheses have been put forth 1. The autoreactive T lymphocytes induce inflammatory reaction with high levels of the proinflammatory cytokines IL-1, TNF-α (tumour necrosis factor-α) and INF-γ (interferon-γ) within the islet microenvironment. These cytokines activate the caspase cascade; 2. Apoptosis is induced directly by contact of autoreactive T lymphocytes with β-cells via the perforating system or Fas/Fas ligand interaction. Before the onset of T1D, a chronic atrophic inflammation within the islets of Langerhans is observed histologically, with the participation of T lymphocytes, macrophages, B lymphocytes and dendritic cells. This condition usually evolves over many months or years when patients are asymptomatic and euglycemic. Symptomatic hyperglycemia occurs after a long latency period, reflecting many functioning β-cells that need to be destroyed before the clinical manifestation of disease (Atkinson and Maclaren, 1994). The main autoantibodies detected in patients with T1D are those against GAD65, tyrosyl phosphatase (IA-2), insulin (IAA) and zinc transporter (ZnT8) (Paschou *et al*., 2014). Studies in mice with early presentation of these autoantibodies suggest that proinsulin is the potential primary target. Another important autoantigen is the GAD enzyme and, anti-GAD autoantibodies are found in approximately 70% of the patients with T1D at the time of diagnosis. IA-2 is also an important autoantigen, with approximately 60% of the patients with T1D presenting positive autoantibodies at the time of diagnosis (Elis *et al*., 1998). Autoantibodies to IA-2 usually appear later than autoantibodies to insulin and GAD and are largely related to the disease progression. The zinc transporter (ZnT8) has also been more recently identified as an autoantigen for T1D. Indeed, 60–80% of the newly diagnosed patients show positive ZnT8 autoantibodies. In children monitored from birth for T1D development, it was observed that ZnT8 autoantibodies appear later than autoantibodies to insulin and typically disappear very early, after the clinical manifestation of the disease (Wenzlau *et al*., 2007; Wenzlau *et al*., 2010).

1.3.2 Pathophysiology of type 2 diabetes

T2D is affected both by genetic predisposition and the environment. An individual's predisposition to T2D depends on ethnicity, and family history has a strong genetic basis. Evidences from epidemiological studies indicate that T2D can be avoided by improving the lifestyle (obesity, low physical activity and an unhealthy diet) (Hu *et al*., 2001; Schellenberg *et al*., 2013) (Figure 1.4). A disarranged feedback loop between insulin secretion and its action results in abnormally high blood glucose levels. The pathophysiology of T2D can be divided into an insulin-resistant phase and a β-cell dysfunction phase. Insulin resistance or prediabetes marks the first phase, wherein the β-cells become insulin resistant. The insulin secreted by the β-cells is rendered incompetent due to ineffective triggering of the down-stream signalling cascade. Individuals in this stage have impaired glucose tolerance. As a feedback mechanism, the pancreatic β-cells move into, an exaggerated mode of insulin synthesis and secretion. The overworking phase of β-cells leads to hyperinsulinemia, a characteristic of the prediabetic stage. This is an asymptomatic stage and hence largely goes undiagnosed (Pramanik *et al*., 2018). According to the World Bank income classification in 2019, the low middle income (LMI) countries contribute to more than 50% of such undiagnosed cases, and India ranks second in the list (Saeed *et al*., 2019). The overworking β-cells eventually exhaust themselves in due course of time. This is the stage where β-cell dysfunction begins, and histological studies on cadavers suggest an approximately 50% decrease in β-cell number (Rahier *et al*., 2008; Butler *et al*., 2010).

In a developing nation like India, the sudden urbanisation and socio-economic transitions viz. rural to urban migration, minimalistic exercise regimen, sedentary lifestyle, circadian misalignment etc., have led to an escalation of diabetes prevalence (Dhanaraj, 2016).

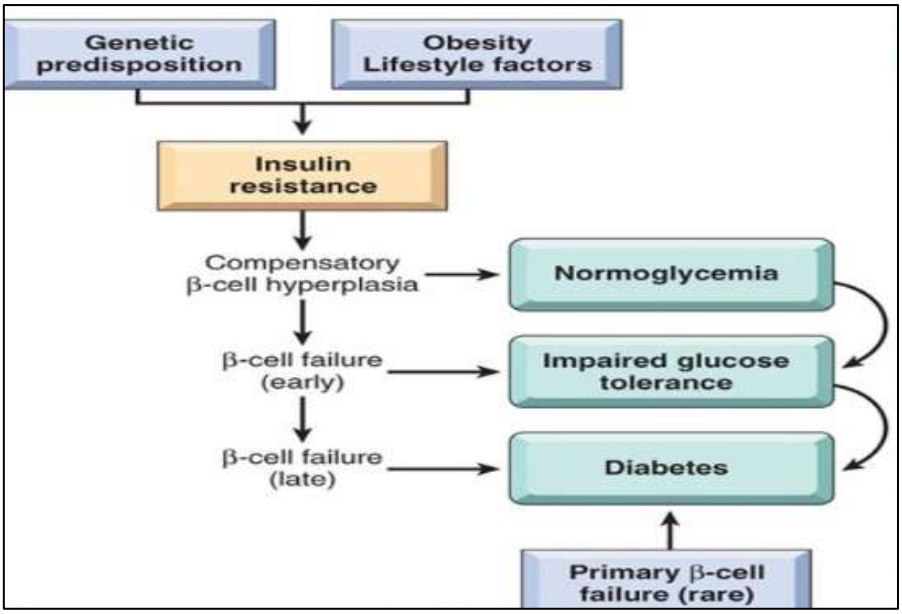

Figure 1.4. Pathophysiology of hyperglycemia in T2D (Lowe, 2005).

1.4 Insulin signalling and insulin resistance

The three best-studied nodes of the insulin signalling pathway are the insulin receptor (IR), insulin receptor substrate (IRS), PI3 kinase (PI3K) and Akt/PKB. Insulin exerts all of its known physiological effects by binding to the IR on target cell plasma membrane (Haeusler *et al*., 2018). It is a hetero-tetrameric receptor tyrosine kinase formed from two extracellular α subunits, which bind insulin, and two membrane-spanning β subunits, each of which contains a tyrosine kinase domain (Hubbard, 2013). There are two IR isoforms, A and B, but the B isoform is much more specific for insulin and is the primary isoform expressed in differentiated liver, skeletal muscle and white adipose tissue (WAT). It thus mediates most metabolic effects of insulin (Belfiore *et al*., 2017). IR has two insulin binding sites but exhibits negative cooperativity, i.e., insulin binding at one site decreases its binding affinity on the other site (De Meyts, 2008). The binding of insulin to IR induces a conformational change in the β subunit, releasing the cis-autoinhibition in the kinase activation loop. This permits trans-autophosphorylation of the activation loop tyrosines, Tyr1162, Tyr1158, and Tyr1163 sequentially (Hotamisligil *et al*., 1994; Wei *et al*., 1995). The β subunit, thus activated by transphosphorylation, undergoes further tyrosine phosphorylation on residues including Tyr972 in the juxtamembrane region resulting in the recruitment of IRS (Youngren, 2007). Most insulin effects are mediated through IRS-1, -2, and src homology and collagen protein (SHC) (White, 2003; Taniguchi *et al*., 2006; Versteyhe *et al*., 2010). The IRS central and C-terminal regions contain 20 potential phosphorylation sites, which on phosphorylation by IR binds to signalling proteins containing SH2 domains. The two main pathways of

insulin signalling from the insulin receptor-IRS node are the phosphatidylinositol 3-kinase (PI3K, a lipid kinase)/AKT (also known as PKB or protein kinase B) pathway (Shepherd *et al*., 1998; Cantley, 2002) and the Raf/Ras/MEK/MAPK (mitogen-activated protein kinase, also known as ERK or extracellular signal-regulated kinase) pathway (Avruch, 2007). Most metabolic effects of insulin are driven by the PI3K pathway and are connected exclusively through the IRS. The PI3K pathway gets activated by the binding of p85 or p55 regulatory subunit of PI3K (an adapter that has eight isoforms) with IRS1 and-2. This results in the activation of the p110 catalytic subunit (which has three isoforms) and the generation of phosphatidylinositol-3,4,5-triphosphate (PIP3), leading to the activation of three isoforms of AKT/PKB by PDK (phosphoinositide-dependent protein kinase) 1 and -2. The PDKs bind to PIP3 in the cell membrane and thereby get activated (Shepherd *et al*., 1998; Cantley, 2002; Taniguchi *et al*., 2006). Four of the critical downstream substrates of AKT/PKB are mTOR [mammalian target of rapamycin, involved in the regulation of protein synthesis (Harris, 2003)]; GSK3 (glycogen synthase kinase 3), involved in the regulation of glycogen synthesis (Cohen, 2001); FoxO (forkhead box-containing protein, O subfamily) transcription factors, especially FoxO1, involved in the regulation of gluconeogenic and adipogenic genes transcription (Accili, 2004) and AS160 (AKT substrate of 160kDa), involved in glucose transport (Sano, 2003). mTOR is a serine/threonine kinase that acts as a nutrient sensor. It is the catalytic subunit of two structurally distinct complexes, mTORC1 and mTORC2. mTOR stimulates protein synthesis by the phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and p70 ribosomal protein S6 kinase (p70S6K). AS160 is a GTPase-activating protein that on phosphorylation activates small G proteins called RAB that are involved in membrane trafficking of glut 4 storage vesicles (GSVs), by blocking the exchange of GTP for GDP. GSVs contain glut 4 (glucose transporter) which are redistributed on the plasma membrane in response to insulin, facilitating the uptake of glucose from circulation (Huang and Czech, 2007) (Figure 1.5).

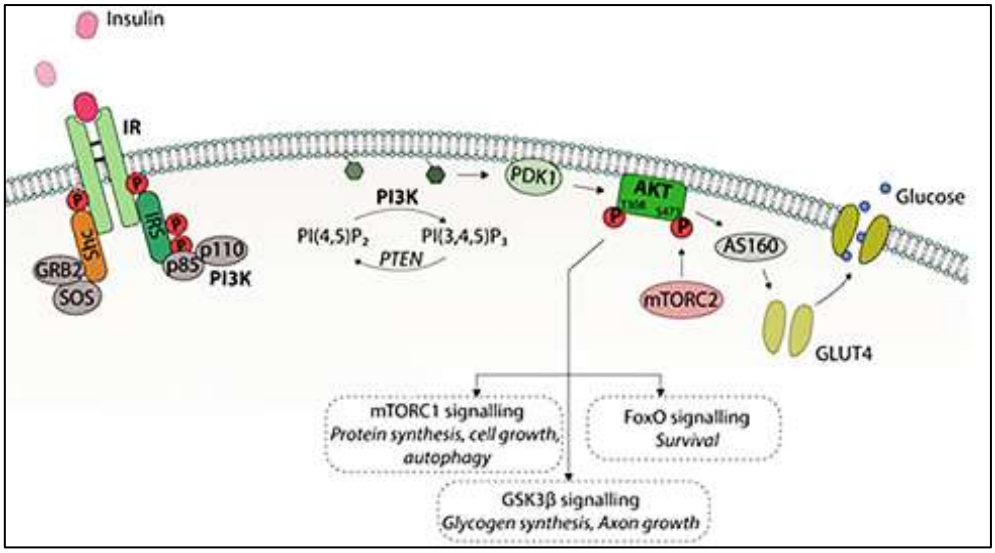

Figure 1.5. Insulin signalling pathway (Gabbouj *et al*., 2019).

Various mechanisms exist to attenuate or terminate the signal induced by insulin, both at the receptor and post-receptor levels (Taniguchi *et al*., 2006). They can range from genetic alterations to inhibitory phosphorylation of IR or downstream signalling molecules. The functional consequences can be impaired IR synthesis, impaired transport to the plasma membrane, impaired insulin binding, impaired transmembrane signalling or impaired endocytosis, recycling and degradation. Moreover, serine hyperphosphorylation at Ser312 marks IRS1/2 for degradation, reducing the IR-mediated signalling relay (Taniguchi *et al*., 2006).

1.5 Etiological factors leading to T2D

T2D is a complex multifactorial and polygenic disorder. Demographic transitions, nutrition and lifestyle in the backdrop of genetic predisposition have been recognised as the main factors responsible for the rising cases of obesity-associated diabetes amongst south Asians (Misra and Shrivastava, 2013). In this context, lifestyle, obesity, ER stress, oxidative stress, mitochondrial dysfunction, and genetic variants have all been envisaged to contribute to the disease pathophysiology.

1.5.1 Lifestyle: Recent studies indicate that metabolic diseases risks are also associated with stress, insomnia, nutritional status, sedentary behaviour etc. Stress affects glucose metabolism through the hypothalamic-pituitary-adrenal (HPA) axis as its chronic activation affects insulin action and may cause insulin resistance and β-cell dysfunction (Harris *et al*., 2017). Insomnia stimulates appetite-regulating hormones and is also associated with increased blood pressure and sympathetic nervous system activities. All of the above factors lead to insulin

resistance and T2D (Li *et al*., 2016). While a high-calorie diet leads to obesity and insulin resistance, many clinical studies have also shown the importance of adequate intake of micronutrients such as vitamin K (Li *et al*., 2018), vitamin D (Lu *et al*., 2018) and magnesium (Barbagallo *et al*., 2015). Antioxidants such as β-carotene and α-tocopherol confer protection against T2D. Various animal-based studies suggested that insulin-induced total peripheral glucose disposal occurred predominantly in muscles (Arnlov *et al*., 2009). Studies have also shown a weakened OXPHOS in the muscle of rats led to significant increase in plasma triglycerides uptake during contractile activity as compared to other muscles. This brings us to conclude that physical activity is required to maintain the energy balance and lack of the same predisposes to metabolic diseases (Hamilton *et al*., 2014).

1.5.2 Obesity: Overweight and obesity are defined by an excess accumulation of adipose tissue (AT) to the extent that impairs both physical and mental health and well-being (Naser *et al*., 2006). Interestingly, both T2D and obesity are associated with insulin resistance. The distribution of body fat is an essential determinant of insulin sensitivity. Individuals with increased visceral or central or abdominal fat have compromised insulin sensitivity. The differential correlation is due to differential relation of AT with genes that make proteins involved in the energy production pathway. Besides being the store house of energy, AT also plays a prime role in metabolism by secreting adipokines, glycerol and non-esterified free fatty acids (NEFAs). In obese individuals, the secretion of NEFAs is the cornerstone factor affecting insulin insensitivity (Karpe *et al*., 2001) as increased NEFA levels have been observed in T2D and insulin-resistant individuals. Furthermore, β-cells lose their function with the continuous exposure to NEFAs (lipotoxicity), as glucose-stimulated insulin secretion pathway is hampered, and insulin biosynthesis is reduced (Al-Goblan *et al*., 2014). Obesity is comparable to an inflammatory state. In obesity, there is dysfunctional lipid homeostasis with increased circulating triglycerides (TG) and free fatty acids (FFA) levels. The excess FFA released from AT by lipolysis works as the primary trigger for macrophage infiltration. These macrophages are of the proinflammatory or M1 phenotype. The M1 macrophages further release monocyte chemoattractant protein-1 (MCP1), bringing about large-scale recruitment of macrophages in AT leading to inflammation and adipose tissue resident macrophage (ATM) polarisation to M1 phenotype (Lee, 2013). In obesity, as the M2 to M1 polarisation occurs, pro-inflammatory adipokines increase, whereas anti-inflammatory adipokines decrease as shown in Figure 1.6.

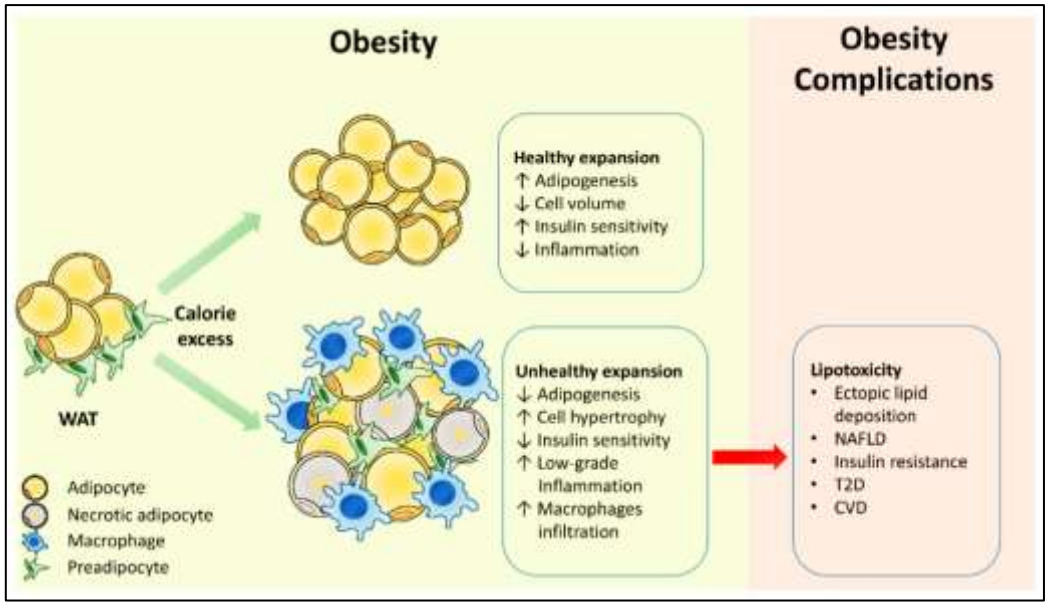

Figure 1.6. T2D pathogenesis in WAT (Longo *et al*., 2019**)**

1.5.3 Adipokines: Adipokines are a large group of >600 bioactive peptide hormones produced by adipose tissue that act locally or distally targeting various organs like brain, liver, pancreas, immune system, vasculature, muscleetc. (Fasshauer M, and Blüher M, 2015). They can broadly be classified into anti-inflammatory and pro-inflammatory. The pro- and anti-inflammatory adipokines synchronise with each other, fine-tuning the various metabolic pathways and managing the systemic inflammatory status (Pramanik *et al*., 2018). Most adipokines show pro-inflammatory activity with exceptions like the adiponectin, secreted frizzled-related protein 5 (SFRP5), visceral adipose tissue-derived serine protease inhibitor (vaspin), and omentin-1. Pro-inflammatory adipokines like leptin, IL1-β, IL-6, TNF-α, resistin. etc. have been associated with insulin resistance (Patel *et al*., 2016; Patel *et al*., 2019; Rathwa *et al*., 2019; Rathwa *et al*., 2020). Among the anti-inflammatory adipokines, adiponectin has potent insulin sensitising property.

1.5.3.1. Adiponectin: It is also known as adipocyte complement-related protein of 30 kDa (147 amino acids) (Acrp30), with an N-terminal collagen-like domain, a C-terminal complement factor C1q-like globular domain, circulating in various polymorphic forms such as trimers, hexamers, and high molecular weight (HMW) globular adiponectin. There are two isoforms of adiponectin receptor, AdipoR1 and AdipoR2, both are structurally related seven-transmembrane receptors. AdipoR1 shows a higher affinity for globular HMW adiponectin and is expressed ubiquitously, but most abundantly in the skeletal muscle. On the other hand, AdipoR2 mainly recognises full-length trimeric adiponectin and is predominantly expressed

in the liver (Yamauchi *et al*., 2003). Another adiponectin receptor that has been identified is called T-cadherin. It acts as a receptor for the hexameric and HMW forms (Hug *et al*., 2004). Adiponectin elicits several downstream signalling events. Adaptor protein phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) acts as a signalling pathway mediator in cross-talk with adiponectin and insulin, and it interacts directly with insulin receptor substrates (Berg *et al*., 2001). Reports from various research groups demonstrate that APPL1 activates AMP-activated protein kinase (AMPK) (Deepa *et al*., 2001; Zhao *et al*., 2015). Upon binding adiponectin to its receptor, APPL1 binds to activate protein phosphatase 2A, resulting in the dephosphorylation of protein kinase Cz (PKCz) rendering it inactive. This, in turn, dephosphorylates liver kinase B1 (LKB1) at its Ser307, allowing LKB1 to translocate from nucleus to cytoplasm, and activate AMPK (Deepa *et al*., 2001). Activation of AMPK is a crucial step in mediating most of the adiponectin effects at the cellular level. AMPK responds to a decrease in cellular energy state by stimulating energy-generating pathways (e.g., oxidation of fats) and inhibiting energy-consuming pathways (e.g., fatty acid, triglyceride, and protein synthesis). Adiponectin drastically increases the expression and activity of PPAR-α, a key transcription factor in metabolic regulation, which in turn upregulates acetyl CoA oxidase (ACO) and uncoupling proteins (UCPs); thereby, promoting fatty acid oxidation and energy expenditure (Yamauchi *et al*., 2003). Interestingly, the action of APPL1 by adiponectin on p38 MAPK (Xin *et al*., 2011) and Rab5, a GTPase downstream of APPL1, improves glucose metabolism in various metabolic tissues (Miaczynska *et al*., 2004) (Figure 1.7). Activated AMPK, in response to adiponectin, is also involved in nitric oxide production through the activation of eNOS, resulting in vasodilation (Cheng *et al*., 2007). Besides, activated AMPK by adiponectin inhibits IKK/NFκB/PTEN triggered apoptosis (Fang *et al*., 2010). As obesity is marked by a rise in proinflammatory factors such as TNF-α, IL-6, ROS, and hypoxia, they suppress adiponectin expression in obese rodents and humans (Li *et al*., 2009). Adiponectin deficient mice develop high fat diet (HFD)-induced inflammation and insulin resistance, whereas exogenous administration of adiponectin or overexpression in transgenic mice results in improved insulin sensitivity (Maeda *et al*., 2002; Kim *et al*., 2007b). Further, LPS-induced TNF-α production in macrophages is dampened by adiponectin through the inhibition of NF-κB activation, and it also stimulates the production of anti-inflammatory IL-10 (Yokota *et al*., 2000; Kumada *et al*., 2004). Besides, adiponectin also increases the differentiation of M2 macrophages and phagocytosis to remove apoptotic cells (Takemura *et al*., 2007). Further, it modulates T cell activation and the inflammatory function of NK cells.

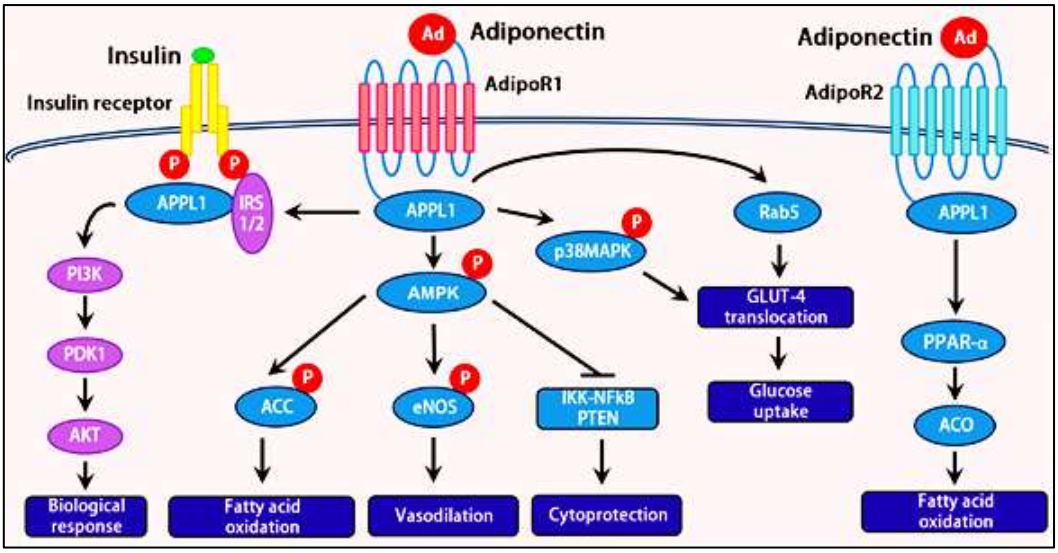

Figure 1.7. Adiponectin signalling pathways (Achari and Jain, 2017)

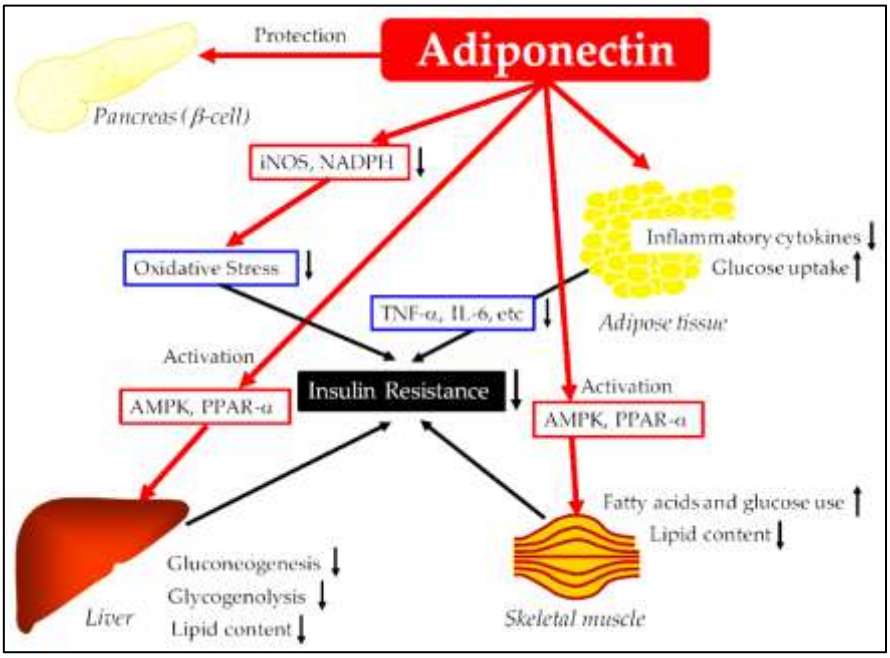

Figure 1.8. Pleiotropic effects of adiponectin (Yanai and Yoshida, 2019)

Overall, adiponectin exerts a protective action on the pancreas and reduces inflammation by inhibiting proinflammatory cytokines in adipose tissue. It also enhances the insulin signalling pathway and brings about glucose homeostasis in the liver and skeletal muscle by activating AMPK and PPAR-α (Figure 1.8). Reduced levels of adiponectin have been documented in obese, insulin-resistant T2D patients.

1.5.4 Genetic alterations: South Asia makes up a quarter of the world's population, harbouring the highest number of T2D cases (Basnyat *et al*., 2004). Compared to European

people, South Asians are at a fourfold higher risk of T2D (Ramachandran *et al*., 2010; Chambers *et al*., 2000). Although all the above mentioned non-genetic factors play a prominent role in developing T2D, disease susceptibility varies between individuals making genetic factors an obvious causative factor for the heritability of T2D ranging from 20%-80%. By the year 2000, numerous candidate genes and linkage studies had been carried out, but could identify only a few susceptibility loci. With genome-wide association studies (GWAS) picking up the trend by 2016, close to ~153 single nucleotide polymorphisms (SNPs) were identified mapping to >120 loci viz. *ADIPOQ, IRS, GCKR, SREBF1, IGF2BP2, CDKAL1* (McCarthy and Zeggini, 2009; Ali, 2013; Wang *et al*., 2016). Certain members of the adipokine family, namely adiponectin, resistin, omentin and leptin are important mediators of inflammation and glucose metabolism. SNPs in these genes have been reported to be involved in the pathogenesis of T2D.

SNPs may be present in the coding or non-coding region of the gene. Interestingly, SNP located in the non-coding region can affect protein synthesis as much as one at the coding region. Interestingly, a significant number of patients depending on their genetic makeup show or eventually develop resistance towards pharmacological interventions. Also, not all T2D patients show the same responsiveness to particular drug treatment. GWAS paved the way for pharmacogenomics which has taken the spot light (Mannino and Sesti, 2012; Becker *et al*., 2013). Thus, the genotype generated by the presence of a SNP needs to be validated for a phenotypic change. Hence, this warrants significant efforts to identify common genetic variants underlying the T2D risk in individuals of south Asian ancestry.

1.5.5 Mitochondrial dysfunction:

Mitochondria play a crucial role in energy metabolism by generating most of the energy used by cells and it was found that the metabolism of both glucose and fatty acids by skeletal muscle is impaired in T2D (Kelley and Simoneau, 1994; Kelly *et al*., 1996). Also, a reduction in the activity of critical enzymes involved in the oxidative pathways was observed in skeletal muscle obtained from individuals with obesity and T2D. It correlated with the severity of insulin resistance (Simoneau and Kelley, 1997). These findings raised the possibility of impaired mitochondrial function as an additional aspect of insulin resistance. It was then postulated that impaired fat oxidation by mitochondria could lead to insulin-resistance by accumulating lipid intermediates (Schmitz *et al*., 1999; Kelley and Mandarino, 2000). In 2002, Kelley and the group further measured the mitochondrial size and found that skeletal muscle mitochondria were smaller in T2D and obese subjects than in muscle from lean

volunteers (Kelley *et al*., 2002). Mitochondrial dysfunction can be a consequence of decreased mitochondrial biogenesis, mitochondrial content and/or a decrease in the protein content and activity of oxidative proteins per unit of mitochondria. All these changes would lead to a reduction in substrate oxidation. The reduced oxidation of lipid, in particular, would lead to the accumulation of metabolically active lipid mediators such as diacylglycerols (DAG) and ceramides (CER). Both these lipid mediators have been shown to inhibit insulin signalling, DAG through protein kinase C activation translocates to the plasma membrane and inhibits insulin receptor (Samue *et al*., 2010), and CER through an inhibition of the protein kinase AKT (Schmitz *et al*., 1999; Bruce *et al*., 2012). Further, decreased ADP-stimulated respiration, ETC complex I and III activities, and mitochondrial density have also been reported (Yokota *et al*., 2009). Obesity is also characterised by increased circulating free fatty acids and accumulation of triacylglycerol that contributes to lipotoxicity, elevated oxidative stress, and impaired energy substrate metabolism and oxidative phosphorylation (OXPHOS) (Bonen *et al*., 2004; Yang *et al*., 2009) (Figure 1.9). Mitochondrial dysfunction is not limited to skeletal muscle alone. It is also found in other organs, viz. liver, β-cells, adipocytes etc. (Pinti *et al*., 2019).

Hepatic dysfunction in nonalcoholic fatty liver disease (NAFLD) is commonly observed in patients with T2D (Petersen *et al*., 2005; Roden, 2006; Masuoka *et al*., 2013; Targher and Byrne, 2013). Alterations in hepatic energy substrate metabolism and mitochondrial function in T2D patients with NAFLD are well characterised. Decreased insulin sensitivity of the liver accompanied by increased hepatic fat storage are two significant metabolic changes found in diabetes patients (Koska *et al*., 2008; Petersen *et al*., 2005; Schmid *et al*., 2011). Mitochondrial intrinsic perturbations in obese, insulin-resistant patients with nonalcoholic steatohepatitis (NASH) include lower maximal respiration, increased mitochondrial uncoupling, and increased proton leak (Koliaki *et al*., 2015). These findings are further strengthened by the observation of decreased ATP content in the T2D liver (Szendroedi *et al*., 2009; Schmid *et al*., 2011).

In the setting of T2D, chronic exposure to hyperglycemia and hyperlipidemia impairs β-cell function. Multiple groups have highlighted mitochondrial structural and functional abnormalities as critical factors in this impairment (Anello *et al*., 2005; Dlasková *et al*., 2010). Pancreatic β-cells from diabetic patients were shown to have increased ETC complexes I and V through decreased ATP levels and ATP/ADP ratio due to increased uncoupling protein 2 (UCP-2) expression in diabetic islet cells with decreased GSIS (Anello *et al*., 2005). Further, increased ETC derived reactive oxygen species (ROS) in high-glucose-

treated MIN6 β-cells was shown to contribute to decreased glucose-induced insulin secretion (Sakai *et al*., 2003). The vital role of mitochondria in optimal β-cell function was further evidenced by the age-related loss of mitochondrial DNA (mtDNA) corresponding with declining insulin secretion (Cree *et al*., 2008; Nile *et al*., 2014).

Further, many ETC components expression decreases in the visceral adipose mitochondria of women with T2D (Dahlman *et al*., 2006). This is also supported by work showing reduced expression of OXPHOS genes in adipose tissue of T2D patients (Nilsson *et al*., 2014). However, others have provided data to support the notion that mitochondrial dysfunction is only present in obese T2D patients (Chattopadhyay *et al*., 2011).

From the above it can be concluded that mitochondrial-targeted therapeutics might be a viable treatment strategy to improve β-cell function in T2D patients.

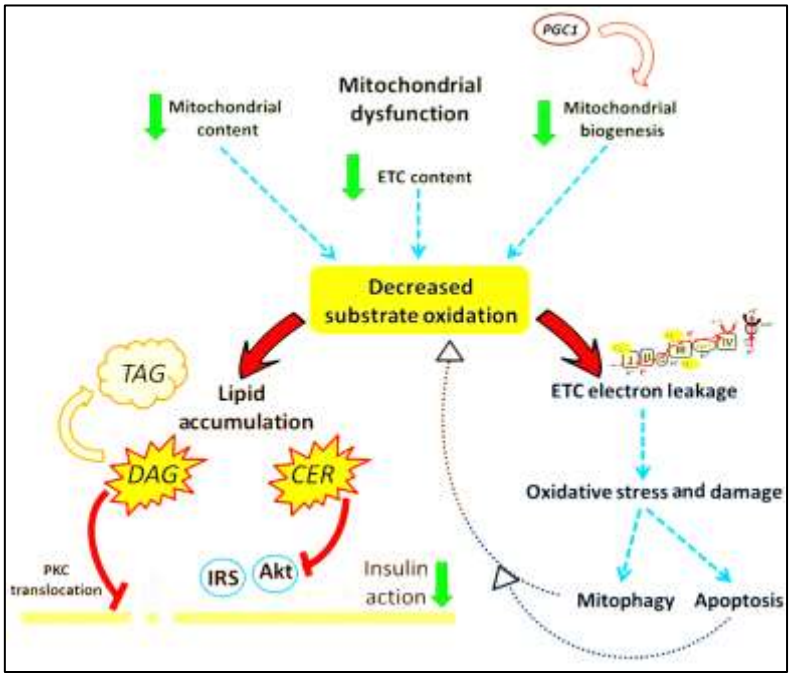

Figure 1.9: Mitochondrial dysfunction in T2D (Longo *et al*., 2019**)**

1.6 β-cell dysfunction and insulin resistance:

The pancreas is about 6 inches long, located behind the stomach. It serves both exocrine and endocrine functions. The exocrine part secretes pancreatic juice containing bicarbonates that neutralise the acid entering the duodenum from the stomach, and digestive enzymes which break down carbohydrates, proteins and fats. The endocrine part (islets of Langerhans) secretes hormones working antagonistically to regulate blood glucose levels and accounts for about 2% of the pancreas. It is made up of α-, β-, δ-, ε- and PP cells which secrete glucagon, insulin, somatostatin, ghrelin and pancreatic polypeptide respectively (Xavier, 2018;

Mandarim-de-Lacerda, 2019). Postprandial blood glucose levels when increase, the GLUT2 transporters transport glucose into the β-cells where it gets oxidised to produce ATP. This results in the closure of K_{ATP} channels (ATP-sensitive), leading to depolarisation of the plasma membrane resulting in release of calcium through the voltage-gated calcium channels, and insulin secretion, as shown in Figure 1.10 (Chen *et al*., 2017).

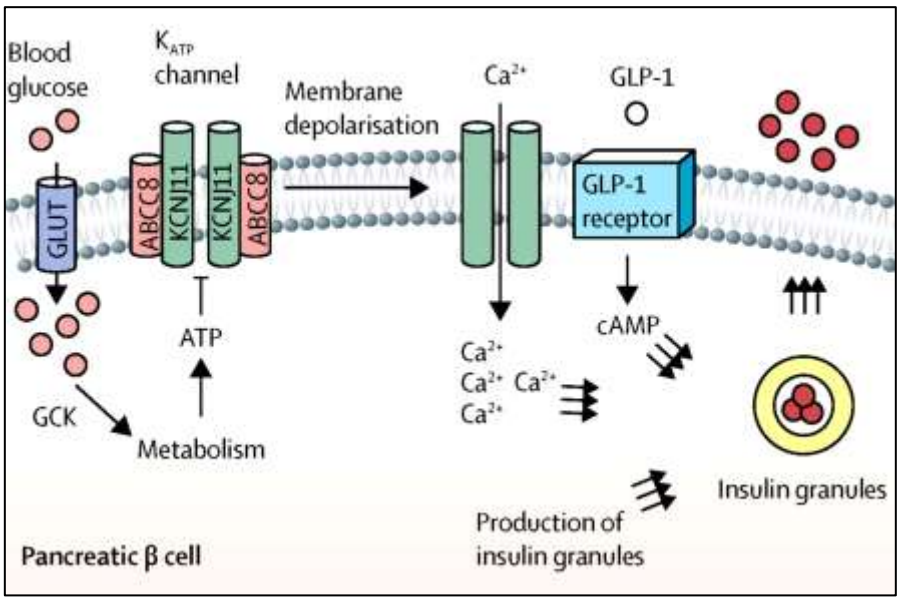

Figure 1.10: Glucose stimulated insulin secretion (Langenberg *et al*., 2013).

Under conditions of excessive nutrition such as obesity, hyperglycemia and hyperlipidemia favouring insulin resistance and chronic inflammation, β-cells exhibit ER stress, oxidative stress, and amyloid stress, eventually leading to loss of islet integrity (Christensen, 2019). An excess of FFAs and hyperglycemia induces ER stress by activating the apoptotic unfolded protein response (UPR) pathways (Yamamoto *et al*., 2019). Stress derived from high levels of saturated FFAs can activate the UPR pathway by several mechanisms, including inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) responsible for ER Ca^{2+} mobilisation; activation of IP3 receptors or direct impairment of ER homeostasis. Besides, sustained high blood glucose levels increase proinsulin biosynthesis and islet amyloid polypeptides (IAAP) in β-cells, leading to the accumulation of misfolded insulin, IAAP and ROS (Yamamoto *et al*., 2019). These effects lead to increased Ca^{2+} mobilisation from ER and favour proapoptotic signals, proinsulin mRNA degradation, and induce interleukin IL 1-β release which results in macrophages enhancing local islet inflammation (Halban *et al*., 2014) (Figure 1.11).

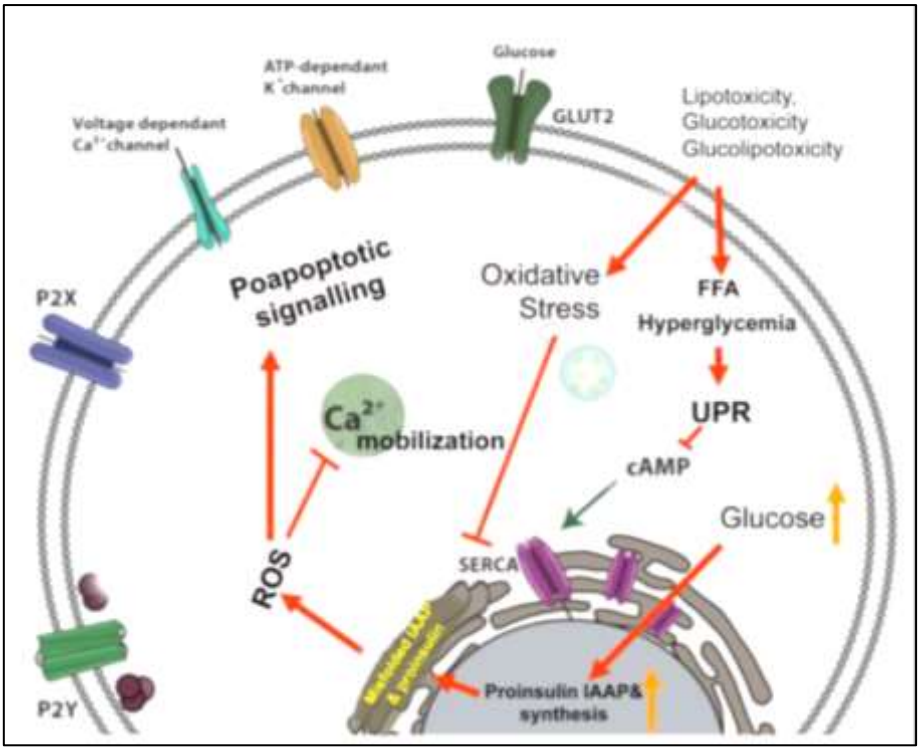

Figure 1.11: Mechanism of β-cell dysfunction (Galicia-Garcia *et al*., 2020)

DNA fragmentation estimation by immunocytochemical techniques is one of the methods for monitoring apoptosis. Terminal deoxynucleotydyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) is one such method but with deficiencies such as labelling of non-apoptotic nuclei due to active gene transcription or strand-breaks as a result of protease digestion, fixation or processing procedures (Duan *et al*., 2003; Rhodes, 2005). Visualizing the translocation of apoptosis inducing factor (AIF) is another method of measuring apoptosis. AIF is a mitochondrial oxidoreductase that participates in cell death programmes and in the assembly of the respiratory chain. Under physiological state, AIF is localized in mitochondria playing a role in various catabolic and anabolic processes. However, in diseased state AIF translocates to nucleus leading the cell into apoptosis (Bano and Prrehn, 2018).

1.7 β-cell regeneration

A 70~100% and 0~65% deficit in β-cell mass was observed in T1D and T2D cadavers, respectively (Butler *et al*., 2003; Matveyenko *et al*., 2008). Thus, β-cell regeneration could be a potential therapeutic strategy for reversing DM. Regeneration of β-cells occurs through either endogenous regeneration or exogenous supplementation by transplantation of cadaveric islets or grafting newly synthesised β-cells *in-vitro*. Numerous strategies and

technologies for constructing insulin-secreting human cells have been developed viz., *in-vivo* stimulation of existing β-cell replication (proliferation), and reprogramming of other pancreatic cells to differentiate into β-cells (neogenesis and transdifferentiation) (Zhou *et al*., 2018). However, the clinical application remains a challenge.

1.7.1 β-cell proliferation:

During foetal development, β-cells are mainly generated from endocrine progenitor cells by differentiation (Finegood *et al*., 1995), while in the late gestational and neonatal stages, β-cells are generated by replication or proliferation of existing β-cells (Dor *et al*., 2004; Meier *et al*., 2008). The rate at which β-cells proliferate reduces post-weaning, and the renewal capacity of β-cells gets limited with adulthood/ late adolescence. Nevertheless, β-cell mass is very dynamic and is likely to change based on cell number to individual cell volume ratio, body weight, pregnancy etc. (Finegood *et al*., 1995; Montanya *et al*., 2000).

Irs–Pi3k–Akt, Gsk3, mTor, ChREBP/cMyc, Ras/Raf/Erk, and Nfats are the major mitogenic signalling pathways mediating β-cell replication. These involve a mitogen upstream activating the downstream signalling pathways viz., nutrients (glucose), epidermal and platelet-derived growth factors, incretins like glucagon-like peptide 1 (GLP-1), and hormones (leptin, estrogen, prolactin, and progesterone). Mitogenic signals stimulate quiescent β-cells to re-enter the cell cycle by regulating the expression of downstream cell cycle regulators such as cyclins, cyclin-dependent kinases (Cdks), cell-cycle inhibitors, and E2F factors (Cozar-Castellano *et al*., 2006; Kulkarni *et al*., 2012; Rieck *et al*., 2012; Bernal-Mizrachi *et al*., 2014; Stewart *et al*., 2015). Pdx1, insulin and glut2 are the critical markers of β-cells (Weinberg *et al*., 2007) (Figure 12). The presence of Ki67 or incorporation of 5-bromo-2'-deoxyuridine (BrdU) and the specific markers of β-cells indicate β-cell proliferation.

1.7.2 β-cell neogenesis:

Neogenesis is defined as the generation and migration of cells from other non-endocrine pancreatic regions, or stem/progenitor cells that can express and secrete insulin in response to a glucose challenge. Lineage tracing studies have shown the pancreatic ductal epithelium to be a potential progenitor of the islet and acinar tissues post-birth (Bonner-Weir *et al*., 2004). Post partial pancreatectomy, the regeneration foci comprise new ductal cells expressing embryonic pancreatic epithelium markers, Pdx1, Hnf6, Foxa2, Tcf1/2, Ngn3 and Sox9, resulting in the formation of new pancreatic lobes (Li *et al*., 2010). Also, Ngn3-positive pancreatic cells act as endocrine progenitors in response to pancreatic injury and give rise to

all islet cell types, including glucose-responsive β-cells via the notch signalling pathway (Apelqvist *et al*., 1999; Gu *et al*., 2002; Xu *et al*., 2008) (Figure 1.12). On the contrary, there are studies suggesting the absence of neogenesis. Overall, neogenesis is suggestive of being either difficult to activate or a rare event (Menge *et al*., 2008; Cavelti-Weder *et al*., 2013; Rankin *et al*., 2013).

1.7.3 β-cell transdifferentiation:

In transdifferentiation, mature cells retrace back to their progenitor form and differentiate back into a new cell type. β-cell regeneration by transdifferentiation occurs from the reversion of exocrine and endocrine pancreatic cells and has been well-characterised. Genetic lineage tracing results in β-cell ablated mouse suggesting the formation of new insulin-producing β-cells from the conversion of α or δ cells (Thorel F *et al*., 2010; Chera *et al*., 2014). The inherent pancreatic lineage and genetic makeup of pancreatic progenitor cells allow transdifferentiation to occur by a much less complex process, therefore providing a more feasible route towards β-cell regeneration, which could be translated into a clinical application in humans (Kim *et al*., 2016). Differentiation of pancreatic lineage is regulated sequentially and regionally by a wide array of transcription factors (Conrad *et al*., 2014). Expression of the specific combination of Ngn3, Pdx1 and Mafa reprogram pancreatic exocrine cells into insulin-expressing cells similar to β-cells in adult mice (Zhou *et al*., 2008). It was demonstrated by Collombat *et al*. (2009) that Pax4 could induce transdifferentiation of α cells into β-cells resulting in glucagon deficiency, which then stimulates the continuous neogenesis (Collombat *et al*., 2009). They further reported that Ngn3 was re-expressed, and transdifferentiation into β-cells remained continuous as long as Pax4 was present. Pax4 acts as an antagonist repressing the homeobox Arx, specific to pancreatic α cells (Collombat *et al*., 2009). In 2013, Courtney and colleagues reported that the selective inhibition of Arx in α-cells is sufficient to promote transdifferentiation into β-cells, regardless of age (Courtney *et al*., 2013) (Figure 1.12).

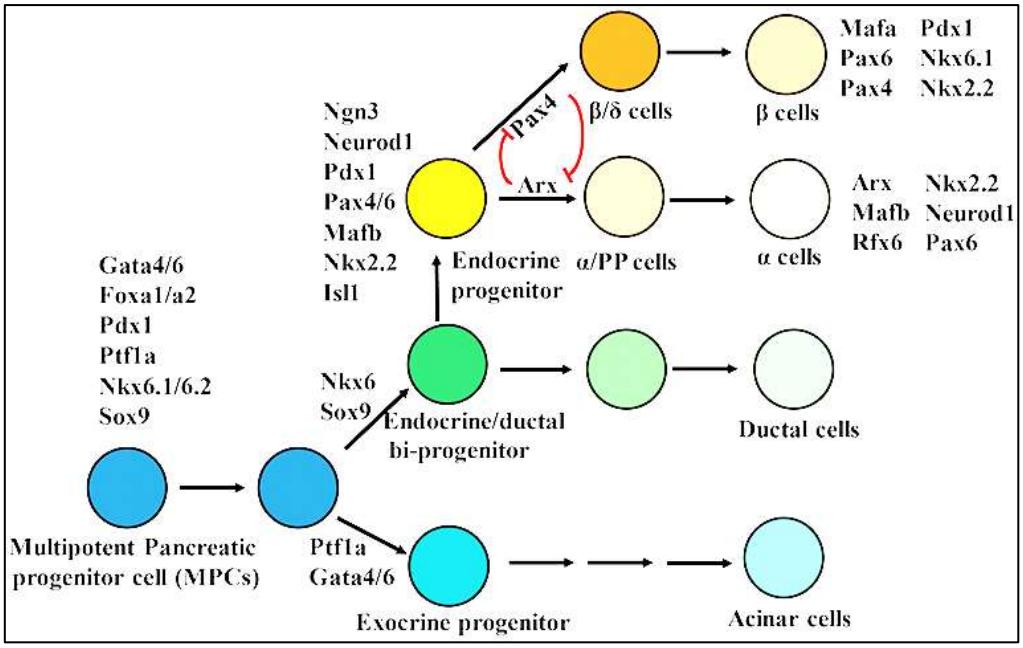

Figure 1.12: β-cell regenerative lineages (Zhong and Jiang, 2019)

1.8 Management of type 2 diabetes:

T2D carries significant morbidity and is the leading cause of kidney failure, lower-limb amputations and adult blindness. T2D treatment is often expensive due to its chronic nature and the severity of its complications resulting to be a huge economic burden on the country (Javalkar, 2019).

Several therapeutic classes of non-insulin hypoglycemic agents are commonly used to manage hyperglycemia in T2D (Table 1.1). However, they pose their own challenges. Hence, most often, the first line of management is through calorie restriction (CR) and exercise in borderline cases or prediabetes.

Table 1.1: Overview of currently available noninsulin hypoglycemic agents (Rodbard *et al*., 2009; Phung *et al*., 2010; Pramanik *et al*., 2018)

Group	Class	Generic name	Side effects
Biguanides	Sensitiser	Metformin	GI side effects, megaloblastic anaemia (vitamin B12 deficiency); temporarily discontinue in patients undergoing radiological studies using contrast media
Thiazolidinediones		Rosiglitazone Pioglitazone	Fluid retention, weight gain, bone fractures, a potential increase in MI; use caution if

			liver impairment
Alpha-glucosidase inhibitors	----	Acarbose Miglitol	GI side effects, three-times-daily dosing
Sulfonylureas	Secretagogue	Chlorpropamide Glibenclamide Glimepiride Glipizide Tolazamide Tolbutamide	Hypoglycemia, weight gain
Glinides		Nateglinide Repaglinide	Weight gain, three-times-daily dosing
Exenatide	GLP-1 analog	Byetta	Pancreatitis, GI adverse effects, expensive, lacking long-term safety data, must be injected; risk of thyroid C-cell tumours with liraglutide; use caution in gastroparesis
Dipeptidyl peptidase-4 inhibitors	DPP-4 inhibitors	Sitagliptin Saxagliptin Linagliptin	Pancreatitis, lacking long-term safety data

Interventions involving dietary and physical activity changes are widely used and appear to be the most successful approaches for improving long-term weight maintenance and health status (Dao *et al*., 2004). CR and exercise/physical activity improve FBG levels by attenuating the extent of oxidative stress (Sohal *et al*., 1996). They also enhance insulin signalling pathway, mitochondrial function and biogenesis (Civitarese *et al*., 2007), WAT remodelling and reversing the adipokine profile (Stanford *et al*., 2015; Mottillo *et al*., 2016).
For more advanced stages of T2D, the management becomes more challenging, and over the counter drugs are used to stimulate insulin release, suppress hepatic glucose output or assist glucose disposal. But T2D being a complex disease with no specific treatment algorithm that will be appropriate for all patients, management of the disease remains the only choice. Combination therapy, in this context, has several potential advantages over stepwise treatment, including a multidirectional approach, to reduce clinical inertia and early achievement of glycated haemoglobin goals (Cersosimo *et al*., 2018). Since both T1D and T2D are characterised by eventual β-cell loss, research focuses on regeneration or preservation of β-cell mass. While numerous plant bioactive compounds have been reported to act as the elixir for dying β-cells (Lee *et al*., 2010; Bharucha *et al*., 2012; Chan *et al*., 2012), a handful of current treatment modalities also exist which can address the worsening

condition of β-cell loss with time. A class of enteric hormones, the incretins regulate blood glucose by stimulating insulin secretion from the β-cells (Garber, 2011). The major incretins secreted in response to a rise in glucose levels are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) from endocrine K and L cells, respectively in the small intestine. GLP-1 can activate G protein-coupled receptors on pancreatic β-cells stimulating insulin secretion (Drucker, 2006). GLP-1 also acts on α-cells to inhibit glycogenesis by decreasing the secretion of glucagon.

Furthermore, GLP-1 acts on the central nervous system inducing delayed gastric emptying and a feeling of satiety. Attempts have been made to identify modes of increasing the otherwise short lived endogenous GLP-1 such as dipeptidyl peptidase-4 (DPP-4) or activating GLP-1 receptors by receptor agonist exenatides. However, both classes of drugs have their shortcomings of causing pancreatitis, gastro-intestinal adverse effects, lack of long-term safety data, thyroid cancer etc. (Rodbard *et al*., 2009; Phung *et al*., 2010).

1.8.1 L-glutamine

GLP-1 has many protective effects on the β-cells, including reducing apoptosis and enhancement of β-cell proliferation and neogenesis (Garber, 2011). This reduces the chances of adverse side effects, such as sudden hypoglycemia (Holst, 2007). Studies on T2D individuals have shown increased insulin secretion, and concomitant decreased glucagon secretion upon treatment with GLP-1 receptor agonists (Garber, 2011). As the existing drugs (enhancing or mimicking GLP-1 action) have significant side effects, search for safer ways to fix the entero-insulinar axis needs to be probed. L-glutamine stimulates GLP-1 secretion in human subjects by raising cytosolic Ca^{2+} and cAMP in intestinal L-cells (Tolhurst *et al*., 2011). Interestingly, L-glutamine levels are low in T2D patients (Tsai *et al*., 2012; Chen *et al*., 2019) but high in prediabetic cases (Owei *et al*., 2019), probably as compensation. Glutamine is an α-amino acid required for the biosynthesis of proteins. It is conditionally essential in humans and circulates at the highest concentration of all the amino acids (~0.7 mmol/l blood in the human). The body can usually synthesise sufficient amounts of glutamine, but in some instances of stress, the body's demand for glutamine increases, and glutamine must be obtained from the diet (Lacey *et al*., 1990; Xiao *et al*., 2016). Glutamine is the physiological precursor of arginine for nitric oxide production (NO), which potentiates insulin secretion in β-cells. Furthermore, glutamine is also the primary source of glutamate and is converted to glutathione. Glutathione reduces oxidative stress and inflammatory processes in β-cells (da Silva *et al*., 2008). L-glutamine serves as a fuel in these cells and

tissues. A high rate of glutamine uptake is characteristic of rapidly dividing cells such as enterocytes, fibroblasts and lymphocytes, where glutamine is a precursor of peptides and proteins. It participates in synthesising nucleotides and nucleic acids (Curi *et al*., 2005). L-glutamine treatment is reported to enhance glucose homeostasis (Opara *et al*., 1996; Bakalar *et al*., 2006; Greenfield *et al*., 2009; Molfino *et al*., 2009; Samocha-Bonet *et al*., 2011), increases adiponectin levels (Abboud *et al*., 2019) and corrects lipid profile (Alba-Loureiro *et al*., 2009; Badole *et al*., 2013). Further, in the T2D model, liver infused with L-glutamine was reported to significantly increase L-alanine production (Comar *et al*., 2016). L-alanine exerts insulinotropic effects on β-cells (Dixon *et al*., 2003; Newsholme *et al*., 2006). Since it also acts as an antioxidant, it could reduce oxidative stress in animal models (Tsai *et al*., 2012; Badole *et al*., 2013; Badole *et al*., 2014). Further, it could maintain mitochondrial integrity by reducing cell permeability and cytochrome c levels and increase mitochondrial membrane potential *in-vitro* (Ahmad *et al*., 2001; Safi *et al*., 2015). Taken together, these findings support its positive effect on alleviating mitochondrial dysfunction. However, there are no studies reported to date. Glutamine can serve as both substrate and stimulator of gluconeogenesis and glycogen synthesis based upon the pathological state (Stumvoll *et al*., 1999). A high-fat diet enriched with glutamine seems to have increased insulin-induced glucose uptake *in-vivo* suggesting enhanced insulin signalling in skeletal muscle and reduced hepatic gluconeogenesis, resulting in improved insulin sensitivity (Abboud *et al*., 2019). L-glutamine controls the biosynthesis of IGF2, which is an autocrine regulator of β-cell mass and function. L-glutamine also activates Akt phosphorylation in β-cells leading to its proliferation (Moullé *et al*., 2017). L-glutamine also upregulates Pdx1 (Corless *et al*., 2006). Pdx1 and Pax4 are transcription factors that activate β-cell proliferation and are extremely important for regulating insulin gene expression and maintaining islet identity (Hayes *et al*.,2013; Brun *et al*., 2004). Pdx1, in concert with Ngn3 and Mafa, marks as an important marker of islet neogenesis (differentiation of acinar cells to β-cell cells). At the same time, Pax4 is a marker of transdifferentiation (α-cell to β-cell conversion) (Zhu *et al*., 2015; Zhang *et al*., 2016) (Figure 1.13).

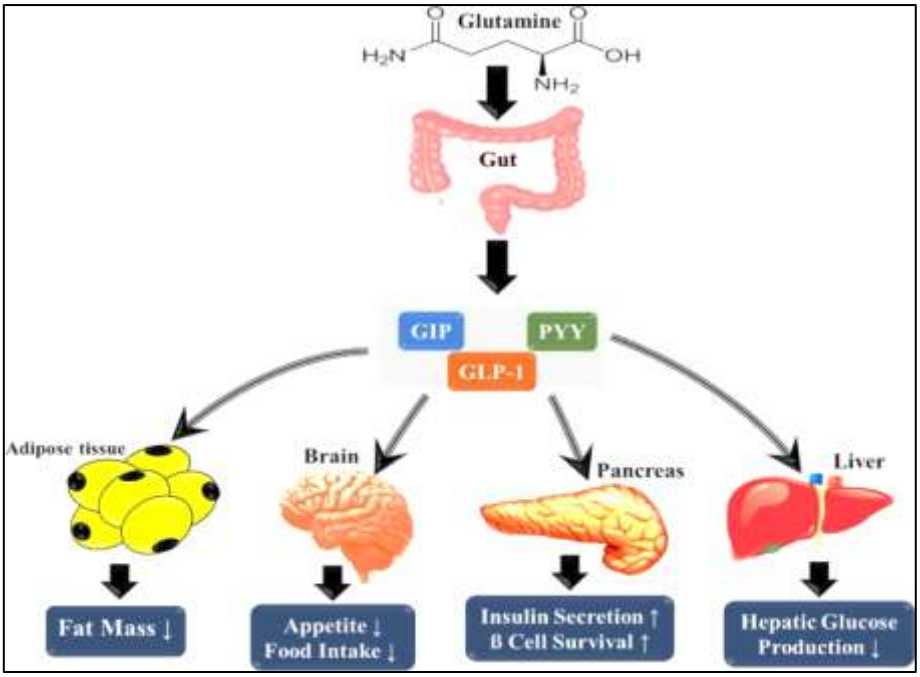

Figure 1.13. The potential effect of L-glutamine supplementation on metabolic variables in diabetes mellitus (Garber, 2011)

1.8.2 Statins:

Obesity/dyslipidemia is associated with reduced anti-inflammatory and increased pro-inflammatory adipokines. As stated earlier, many of these adipokines are crucial in maintaining the status of insulin sensitivity of peripheral tissues, viz. adipose tissue and skeletal muscle (Pramanik *et al*., 2017). As a result of this, regulating hyperglycemia by merely regulating the hepatic glucose output or enhancing glucose clearance seems insufficient. Statins or 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are potent inhibitors of cholesterol biosynthesis. Statins were first identified in fungi as secondary metabolites (Brown *et al*., 1976). ML-236B, one of the first natural HMG-CoA reductase inhibitors, was isolated from *Penicillium citrinum* and was found to be a potent competitive inhibitor of HMG-CoA reductase (Endo *et al*., 1976). Currently, seven statins (atorva-, simva- rosuva, pitava- lova, prava, and fluvastatin) are being used to treat dyslipidemia. Each of the statins is unique in its tissue permeability and pharmacokinetics owing to its hydrophilic or lipophilic nature. Meta- analyses, genetic studies and post hoc analyses have shown positive association of statins with increased risk of new-onset diabetes (NOD), especially in insulin-resistant obese elders (Sattar *et al*., 2010; Chan *et al*., 2011; Waters *et al*., 2013; Ruscica *et al*., 2014). Thus, FDA and EMA changed the labels of all statins in USA and Europe, warning of the possibility of statin-induced NOD or deteriorating

glycemic control in T2D patients. However, recent meta-analyses and retrospective studies affirm that diabetogenicity of statins is dose related with unknown mechanisms. Among the statins, only pravastatin and pitavastatin are reported not to depreciate glycemic parameters either with or without T2D (Arnaboldi and Corsini, 2015). The characteristic structural modification of pitavastatin imparts an improved pharmacokinetic profile to it. It also confers a significant LDL lowering efficacy at much lower doses evading cytochrome P450 mediated metabolism and excretion of most of the bioavailable fractions, thereby allowing reabsorption by the small intestine and enterohepatic recirculation, increasing its bioavailability (Saito, 2011). Further, comparison of the various statins in terms of their adiponectin enhancing ability projects pitavastatin as the best statin, as it could increase adiponectin levels by 27.2±15.9% (Inami *et al*., 2007; Nomura *et al*., 2008; Arao *et al*., 2009; Nomura *et al*., 2009; Matsubara *et al*., 2012; Nomura *et al*., 2012; Kurogi *et al*., 2013). *In vitro* studies suggest that the effect of pitavastatin on adiponectin may be related to the prevention of adipocyte hypertrophy and adipokine dysregulation (Ishihara *et al*., 2010). Statins have been reported to be effective in bringing about glucose homeostasis, increasing adiponectin levels and reducing TG, TC, and LDL in various *in-vivo* studies (Yoshika *et al*., 2010; Matsubara *et al*., 2012; Lee *et al*., 2016; Chen *et al*., 2019; Iwata *et al*., 2019; Cho *et al*., 2020). Moreover, pitavastatin has also been shown to inhibit oxidative stress induced endothelial senescence by phosphorylating Akt at Ser473, leading to increased expression of endothelial nitric oxide synthase (eNOS), SIRT1 and catalase. Due to increased adiponectin levels, pitavastatin treatment seems to have a glucoregulatory effect (Ishihara *et al*., 2010; Cho *et al*., 2020), reduced β-cell apoptosis and increased β-cell regeneration (Zhao and Zhao, 2015) (Figure 1.14).

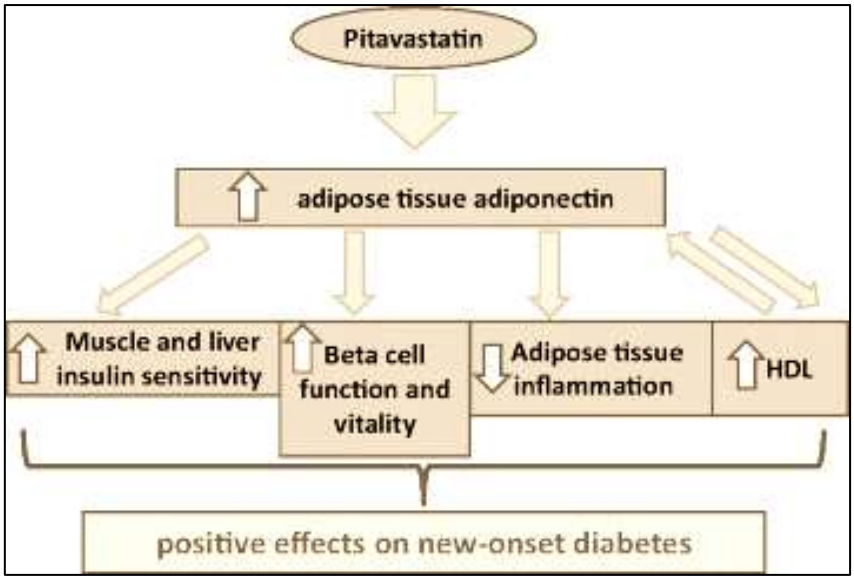

Figure 1.14: Possible mechanisms of the beneficial effects of pitavastatin (Arnaboldi and Corsini, 2015)

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Objectives

I. To evaluate the association of *ADIPOQ* polymorphisms with type 2 diabetes (T2D) in Gujarat population and to study the possible genotype-phenotype correlation with plasma adiponectin levels and metabolic parameters.

1. To study the association of following single nucleotide polymorphisms with T2D
    a) *ADIPOQ* promoter -11377 C/G (*rs266729*)
    b) *ADIPOQ* intron 1 +10211 T/G (*rs17846866*)
    c) *ADIPOQ* exon 2 +45 T/G (*rs2241766*)
    d) *ADIPOQ* intron 2 +276 G/T (*rs1501299*)
2. To estimate plasma high molecular weight (HMW) and total adiponectin levels.
3. To study the possible genotype-phenotype correlation of plasma adiponectin levels and risk towards T2D, and various metabolic parameters.

II. To investigate the therapeutic potential of small molecule enhancers for secretion of adiponectin (pitavastatin) & GLP-1 (L-glutamine) in T2D mouse model.

1. To develop high fat diet (HFD)+ streptozotocin (STZ) induced T2D mouse model.
2. To study the effect of pitavastatin and L-glutamine alone and in combination in HFD+STZ induced T2D mouse model by the following parameters:
    a) To evaluate glucose tolerance and insulin sensitivity.
    b) To estimate plasma insulin and adiponectin levels, and lipid profile.
    c) To study transcript levels of glucoregulatory enzymes and enzyme activities in liver.
    d) To study transcript levels of genes involved in mitochondrial biogenesis and ETC complex activities in skeletal muscle.
    e) To study expression of proteins involved in insulin signalling pathway in skeletal muscle.
    f) To study pancreatic β-cell regeneration and β-cell death.