# Chapter 1

# Introduction and Review of Literature

## 1. Introduction

The incidence of lifestyle diseases like diabetes mellitus, dyslipidemia, overweight/obesity, hypertension and cardiovascular diseases is rising. The prevalence of these diseases has reached alarming proportions, especially among Indians in recent years due to rapid economic development and increasing westernization of lifestyle in the past few decades (Oberoi and Kansra, 2020; Pappachan, 2011).

### 1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated levels of blood glucose. Two primary mechanisms have been proposed to understand the pathogenesis of DM, autoimmune destruction of the pancreatic β-cells resulting in scanty insulin production (type 1 diabetes); and endogenous resistance of body cells to the insulin action (type 2 diabetes) (ADA, 2014). Chronic hyperglycemia in DM leads to several macrovascular (ischaemic heart disease, stroke, and peripheral artery disease) and microvascular (neuropathy, nephropathy, and retinopathy) complications (Chawla et al., 2016).

### 1.2 Classification

There are three main types of DM: type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes (GDM)

a) T1D, also known as "juvenile/childhood-onset diabetes" or "insulin-dependent diabetes", is characterized by autoimmune β-cell destruction, leading to absolute insulin deficiency. The treatment requires regular administration of insulin or its analogues (ADA, 2019). It represents 5-10% of the total number of diabetic cases. The exact cause of T1D is not yet known. However, a complex interaction of environmental and genetic factors leads to the development of T1D in early childhood (WHO, 2016).

b) T2D, also known as "adult-onset diabetes" or "non-insulin dependent diabetes", is characterized by progressive loss of insulin secretion by β-cells leading to insulin resistance (ADA, 2019; WHO, 2016). Thus, T2D is the result of ineffective response of the body to the insulin produced. T2D represents 95% of all diabetic cases. Ethnicity, family history combined with obesity, unhealthy dietary pattern and limited physical exercise are the primary causes of T2D (WHO, 2016). The grey zone of the transition from normoglycemia to DM is often characterized by "Impaired Glucose Tolerance" (IGT) or "Impaired Fasting Glycemia" (IFG). The latter is generally recognized as prediabetes and it is estimated that about 1 out of 4 individuals with IGT/IFG will progress to T2D within a period of 3-5 years

(Nathan et al., 2007). "Prediabetes" is the term used for people with IGT/IFG (IDF Diabetes Atlas, 2019).

c) GDM: It is the condition of elevated blood glucose levels during pregnancy in women without a previous DM history (ADA, 2018). In this case, the infants are at high risk of developing DM later in their adulthood.

## 1.3 Epidemiology

Recent findings suggest that the burden of DM has risen significantly over the past decade and may be considered a growing epidemic. The majority of diagnosed cases lie between the fourth and seventh decade of life (Ogurtsova et al., 2017). Currently, 9.3% of the adult population is diagnosed with DM. The total number is predicted to rise to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045 (IDF Diabetes Atlas, 2019). According to the IDF estimates, the worldwide prevalence of DM is summarised in Fig. 1.1. India ranks second in the world, having 77 million people suffering from DM, and it is estimated that by 2030 and 2045, the numbers will touch 101 million and 134.2 million, respectively, and the number of people having undiagnosed DM is 43.9 million, which accounts for 57% of the population (IDF Diabetes Atlas, 2019). Increased rates of urbanization and socio-economic transitions i.e., rural to urban migration, sedentary lifestyle, and other lifestyle disorders are the main reasons for the regional disparities (Tamayo et al., 2014). While the global prevalence of diabetes in urban areas is 10.8%, in rural areas, it is lower, at 7.2%. However, this gap is closing, with rural prevalence rising (IDF Diabetes Atlas, 2019). Currently, the number of people with T1D is 4.56 billion (0-19 years). However, T2D often remains undiagnosed, hence no reports show its true prevalence (WHO, 2016). In 2017, the cases of DM diagnosed in women aged 20-79 years were 8.4% compared with 9.1% observed among men, and it is expected that the percentages will rise to 9.7% and 10%, respectively (Ogurtsova et al., 2017).

The increasing prevalence of diabetes worldwide is driven by a complex interplay of socioeconomic, demographic, environmental and genetic factors. The continued rise is primarily due to an upsurge in T2D and related risk factors, including rising levels of obesity, unhealthy diets, and widespread physical inactivity. Also, the levels of childhood-onset T1D are also on the rise.

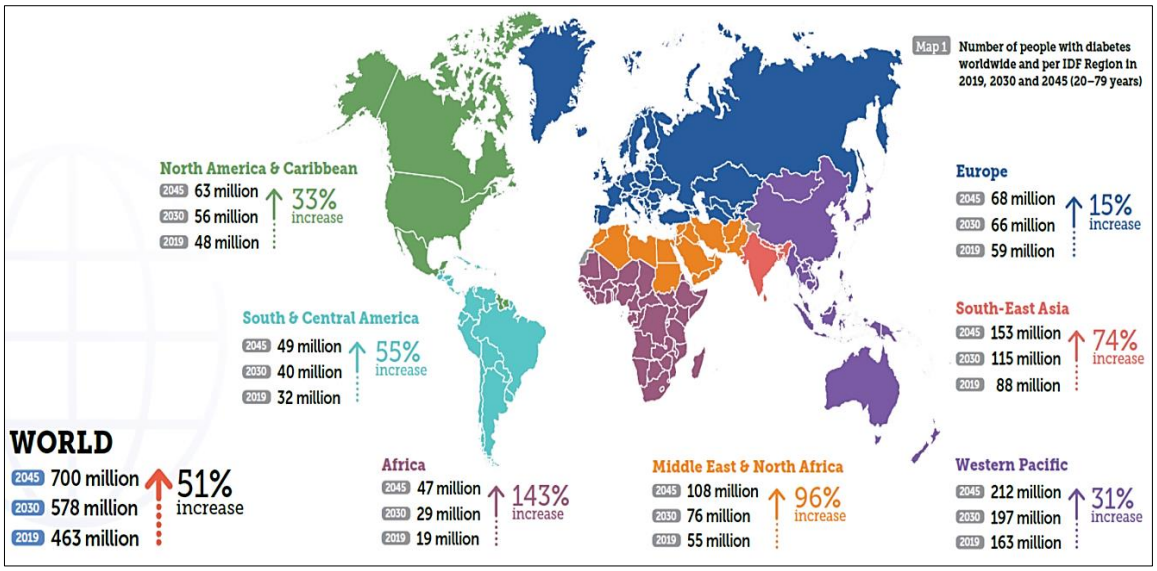


**Figure 1.1 Worldwide prevalence of diabetes mellitus.** (IDF Diabetes Atlas, 2019)

## 1.4 Diagnostic Criteria for Diabetes Mellitus

Prediabetes and DM can be diagnosed through blood tests by monitoring fasting blood glucose levels (FBG) after eight fasting hours. The glycated haemoglobin (HbA1c) provides an estimate of the previous three months' blood glucose levels. Currently, the WHO and IDF recommend a two-hour oral glucose tolerance test (OGTT) to detect IGT and IFG.

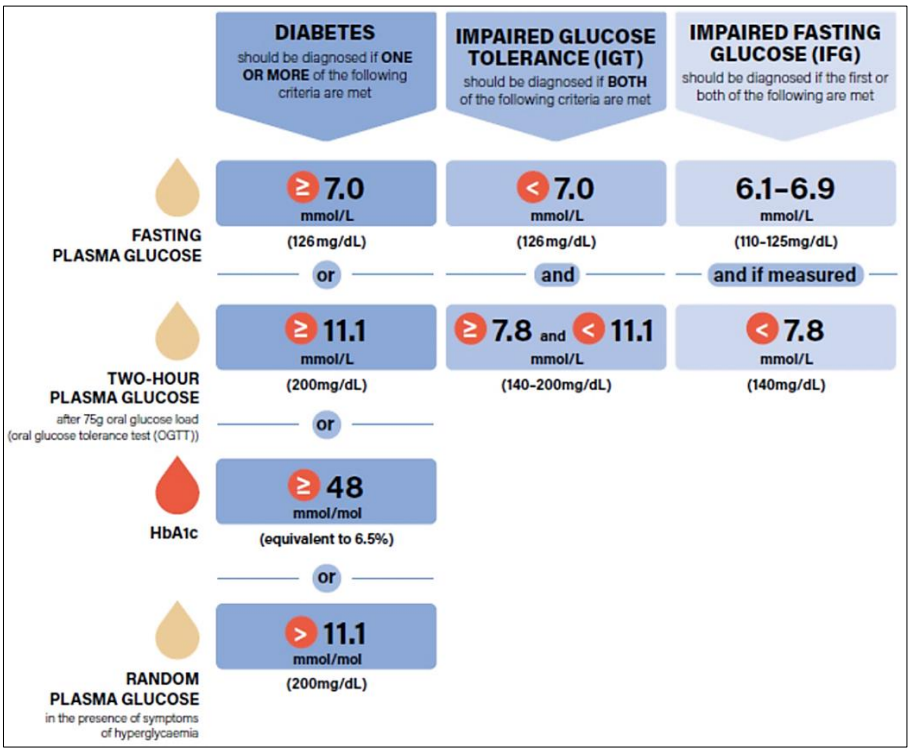


**Figure 1.2 Diagnostic criteria for diabetes mellitus.** (WHO, 2016)

For T1D, in the presence of symptoms (polyuria, polydipsia and unexplained weight loss), the diagnosis becomes possible without OGTT if the following are present; a random venous blood glucose concentration ≥ of 11.1 mmol/l or a fasting blood glucose concentration ≥ of 7.0 mmol/l (whole blood ≥ 6.1 mmol/l or HbA1c ≥ 6.5%). The diagnosis criteria for diabetes, IGT and IFG are summarised in Fig. 1.2.

## 1.5 Pathogenesis of Diabetes Mellitus

Pancreatic β-cell dysfunction and cell death are vital processes in developing T1D and T2D (Cnop et al., 2005). The pathogenesis of T1D and T2D is fundamentally distinct, differentially impacting early β-cell dysfunction (immune-mediated and metabolic in T1D and T2D, respectively) and cell fate (massive versus mild- to- moderate β-cell loss) (Eizirik et al., 2020). Cytokines (IL-1β and IFNγ) can lead to β-cell dysfunction and death in T1D (Ramos-Rodriguez et al., 2019). In contrast, hyperglycemia and free fatty acids (FFAs) might elicit cellular stress (oxidative stress, ER stress, and inflammation), impairing β-cell function and survival in T2D. Understanding the mechanisms behind β-cell failure is critical to prevent or revert DM.

### 1.5.1 Type 1 Diabetes Pathogenesis

T1D is caused by autoimmune-mediated (CD8+ T cells recognizing and targeting specific antigens expressed on the β-cell surface in the context of HLA class I) β-cell apoptosis, and dysfunction leading to the lifelong need for exogenous insulin therapy. T1D is the consequence of a complex interaction between invading or resident macrophages and T cells. The interaction leads to the release of chemokines and cytokines in the islet microenvironment. This delivers cell–cell pro-apoptotic signals, and β-cells via signals generated physiologically (for instance, degradation products of insulin or other components of the β-cell dense core granules) or by stress, injury or dying β-cells, attracts and activate immune cells to the islets (Eizirik et al., 2009; Gonzalez- Duque et al., 2018; Thomaidou et al., 2018). This interaction depends on the host genetic background, age and environmental factors such as viral infections and diet, among others (DiMeglio et al., 2018; Ilonen et al., 2019; Op de Beeck et al., 2016). Pathogenic crosstalk between immune cells and β-cells can trigger local inflammation (insulitis) and progressive β-cell dysfunction and death, mainly via apoptosis (DiMeglio et al., 2018; Eizirik et al., 2009; Todd, 2010). Alternatively, local mechanisms might be arrested that dampen the immune response and restore physiology (Colli et al., 2018; Martinov and Fife, 2020). Some individuals from families affected by T1D show evidence of β-cell dysfunction, such as decreased first phase glucose-stimulated C-

peptide release or increased circulating proinsulin–insulin ratios and absence of β-cell autoantibodies (Sims and DiMeglio, 2019). This observation suggests that β-cell dysfunction could precede the autoimmune assault in T1D or reflect 'scars' of a previous, resolved autoimmune episode. The prevalence of T1D in children is doubling every 25 years (Patterson et al., 2019) and causes an average loss of 11–12 years of life expectancy (Huo et al., 2016). A three-stage classification system has been proposed for T1D. Stage 1 defines the presence of β-cell autoimmunity (i.e., two or more types of autoantibodies) in normoglycemic individuals. Stage 2 marked by dysglycemia (but no overt diabetes mellitus) in the presence of β-cell autoimmunity and stage 3 as clinical T1D (Insel et al., 2015). Presently, no therapeutic approaches exist that prevent or cure T1D (DiMeglio et al., 2018; Greenbaum et al., 2018), although a recent trial in stage 2 patients, using a monoclonal antibody against CD3 (a surface molecule present on CD8+ T cells), delayed, but did not prevent disease onset by ~2 years (Herold et al., 2019).

### 1.5.2 Type 2 Diabetes Pathogenesis

In T2D, relative insulin deficiency due to β-cell dysfunction is a critical factor in developing disease that often coexists with insulin resistance (Cnop et al., 2007; Lyssenko et al., 2005; Weyer et al., 1999). Although T2D represents the bulk (80%) of all DM cases, it remains an ill-defined form of the disease and a diagnosis of exclusion: no specific diagnostic criteria exist for T2D. Clustering approaches using age at diagnosis as well as BMI, HbA1c, HOMA estimates of β-cell function and insulin resistance, and glutamic acid decarboxylase autoantibodies have subtyped patients into moderate or severe forms of T2D, with a predominance of insulin resistance or insulinopenia (Ahlqvist et al., 2018). Obesity, energy-rich 'western' diets, older age and sedentary lifestyle are key risk factors for T2D (Zheng et al., 2018) that have led to a four-fold increase in the number of cases over the last four decades (NCD Risk Factor Collaboration, 2016). These risk factors can precipitate both β-cell failure and insulin resistance. Many drug classes exist to manage T2D, none of which have been shown to modify the progressive decline in β-cell function over time. β-cell insults include cytokine-induced inflammation, obesity, insulin resistance, and overconsumption of saturated fat and FFA. Apart from the loss of β-cell function and mass, islet integrity is also compromised, which could diminish its incretin function, probably due to disturbed cell-cell communication (Halban et al., 2014). The factors implicated in the pathogenesis of T2D are summarized in Fig. 1.3.

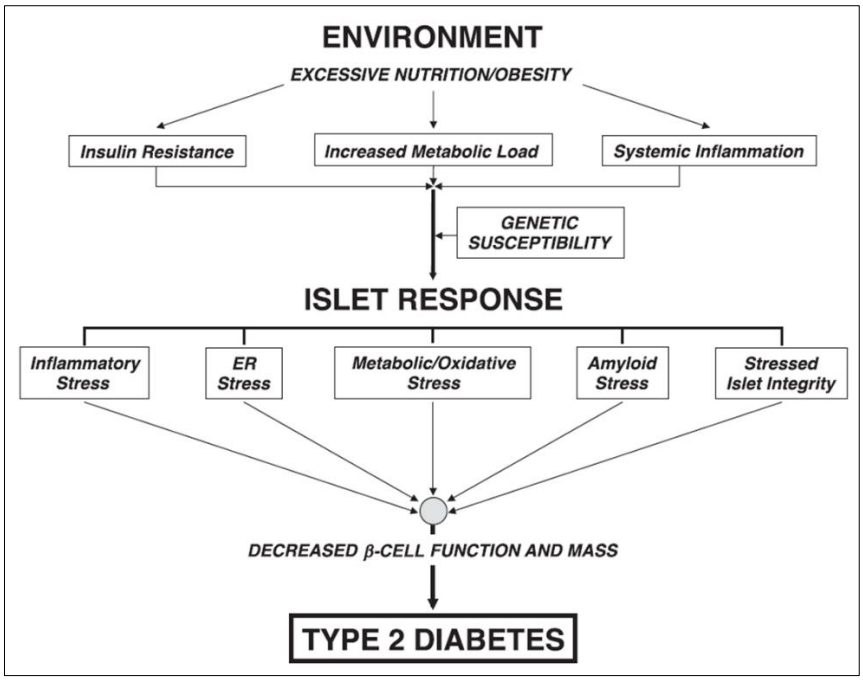


**Figure 1.3 Effect of stressors on the β-cell in the pathogenesis of T2D.** The hypernutritional state in obesity, hyperglycemia and hyperlipidemia leads to an increased metabolic load coupled with insulin resistance and chronic inflammation. The pancreatic islet response to this new environment is likely to be variable among individuals with different genetic susceptibility, including inflammatory stress, ER stress, and metabolic and oxidative stress (e.g., glucotoxicity, lipotoxicity, and glucolipotoxicity), amyloid stress, and loss of islet cell integrity. If untreated, these interrelated stressors could increase with time, promoting β-cell dysfunction (coupled with increased glucagon secretion). Ultimately the loss of β-cell mass and possibly dedifferentiation marks the onset of T2D (Halban et al., 2014).

### 1.5.2.1 Role of Genetic Factors in T2D pathogenesis

An individual's risk of developing T2D is determined by a complex interplay between genetic and environmental/ lifestyle factors. Genotype plays an important role as studies on monozygotic twins showed a 76 % concordance for T2D and a 96 % concordance for impaired glucose tolerance. Furthermore, a family history of T2D doubles an individual's risk of developing the disease (Medici et al., 1999). Epidemiological evidence showed a dramatic increase in T2D over the past 60 years, suggesting that besides genetic factors, alterations in dietary habits, sedentary lifestyle and increased consumption of calorie-rich foods lead to T2D (Scully, 2012). T2D is a polygenic disease, and current evidences favour the idea that in most individuals, the risk of developing the disease is determined by the combination of several genetic variants at multiple gene loci, each of which confer only a marginal increase in disease risk (Lyssenko and Laakso, 2013). Thus, T2D is distinct from monogenic forms of diabetes, such as maturity-onset diabetes of the young (MODY) and neonatal diabetes (Gloyn et al., 2004). As T2D is a polygenic disorder, different combinations of genes in different

individuals result in phenotypic variations. The best method for identifying genes contributing to polygenic diseases is genome-wide association studies (GWAS). These are based on the association of common genetic variants i.e., single-nucleotide polymorphisms (SNPs) with a given phenotype, such as hyperglycemia. To date, more than 70 gene loci are found to be associated with T2D in large-cohort studies (Mahajan et al., 2014), and majority of these loci are implicated in β-cell functioning. GWAS studies involve large cohort size (sometimes >100,000 people) to generate sufficient statistical power. However, as a consequence of GWAS design and the genetic architecture of T2D, causal variants and genes cannot be easily inferred from genetic association studies, hindering the functional interpretation and clinical translation. GWAS is designed to detect SNPs (located in linkage disequilibrium with other variants) that act as a proxy for disease-associated regions or loci, and not necessarily the actual causal variants (Slatkin, 2008). Moreover, association is found in the non-coding regions which influence the disease risk by regulating the genes. The SNPs in non-coding regions are named after the nearest protein coding genes, but this proximity actually does not imply its causality (Maurano et al., 2012). Thus, it is important to find both the gene and a causal variant affecting disease susceptibility. For instance, a causal variant in *melatonin receptor 1B* (*MTNR1B*) gene has been implicated in T2D risk with a functional link. The risk allele (rs10830963 G allele) creates a binding site for the transcription factor NEUROD1 and is associated with preferential binding in human pancreatic β-cells. This event also implicates increased FOXA2-bound enhancer activity and *MTNR1B* expression (Bouatia-Naji et al., 2009). Another meticulous approach revealed the direct influence of a subset of diabetes risk loci on impaired insulin secretion *ex-vivo,* providing mechanistic insights into the role of these genetic variants (Rosengren et al., 2012). High-throughput screens also facilitate the transition from T2D GWAS association signals to individual functional follow-up studies by prioritising candidate causal genes based on functional data (Grotz et al., 2017).

Furthermore, it should be noted that ethnicity greatly influences the distribution of gene polymorphisms and particularly cytokine genes polymorphisms (Hoffman et al., 2002) and thus, association studies must be carried out in different ethnicities to substantiate the association of causal genetic variants with their functional link in disease susceptibility.

#### 1.5.2.2 Cytokines, Oxidative Stress, ER Stress and Inflammation

Obesity leads to inflammation due to secretion of the pro-inflammatory cytokines, leading to insulin resistance. Proinflammatory cytokines cause β-cell death via the induction of mitochondrial stress and other responses (Cnop et al., 2005; Gurgul-Convey et al., 2011). Cytokines secreted by immune cells that have infiltrated the pancreas are crucial mediators of β-cell destruction (Lin et al., 2012). Besides, oxidative stress is thought to be a primary cause of insulin resistance in a hyperglycemic state. Enhanced generation of reactive oxygen species (ROS) and oxidative stress occurs in mitochondria due to an overload of glucose and oxidative phosphorylation. Endoplasmic reticulum (ER) stress also plays an essential role as it is also a source of ROS. The interconnection between organelles through mitochondrial-associated membranes (MAMs) generate ROS in mitochondria promoting ER stress. Therefore, a state of stress and mitochondrial dysfunction are consequences of this vicious cycle. ER stress is also associated with β-cell apoptosis in T2D (Marchetti et al., 2007). Further, the limited glycolytic capacity of β-cells can generate ROS, and the ensuing oxidative stress can uncouple glucose-sensing from insulin secretion (Robertson, 2004). β-cells are highly dependent on ATP production for endogenous incretins that potentiate glucose-stimulated insulin secretion (GSIS) and are vulnerable to excess ROS because of their inherently low expression of antioxidant enzymes (Simmons, 2007). The imbalance or reduced availability of nutrients to β-cells, increased ROS production, lower ATP synthesis, and inadequate antioxidant balance may predispose to β-cell death /dysfunction (Reusens et al., 2011). Hyperglycemia and hyperlipidemia (high FFA), which lead to glucotoxicity and lipotoxicity, have been implicated in the development of cellular stress in β-cells and peripheral tissues. Factors responsible for insulin resistance and β-cell loss are shown in Fig. 1.4.

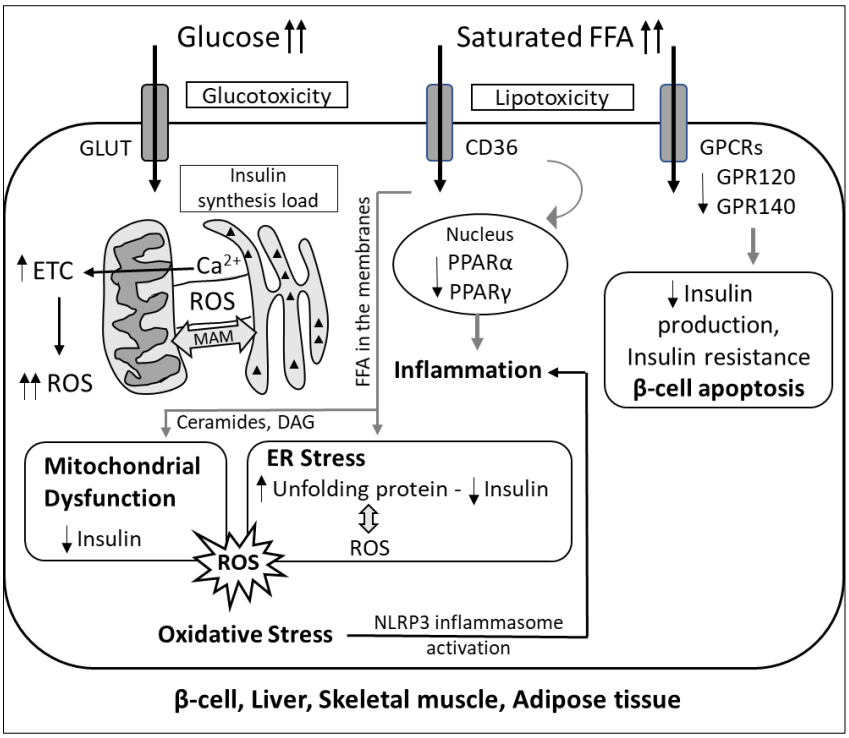


**Figure 1.4 Cellular stress, insulin resistance and β-cell apoptosis.** Glucotoxicity and lipotoxicity lead to ER stress, oxidative stress, and inflammation via ROS generated in the ER and mitochondria. Binding of saturated FFAs to GPCRs results in decreased insulin production and β-cell apoptosis, causing insulin resistance in peripheral tissues (Burgos-Morón et al., 2019).

### 1.5.2.3 Obesity and Insulin Resistance

Macrophage accumulation in obese adipose tissue is typical, and here they secrete pro-inflammatory cytokines that modulate adipose tissue glucose and lipid metabolism (Tateya et al., 2013). The activation status of infiltrating macrophages is vital in the progression of metabolic diseases. Two different polarisation states of macrophages, M1 (pro-inflammatory) and M2 (anti-inflammatory) have been characterized so far. The proinflammatory M1 form is stimulated by pro-inflammatory mediators such as lipopolysaccharide (LPS), tumour necrosis factor-alpha (TNF-$\alpha$), and interferon-gamma (IFN-$\gamma$). These macrophages produce and secrete TNF-$\alpha$, IL-1, and IL-6, enhancing the inflammatory response.

Interestingly, a diet high in lipid content was shown to polarise Kupffer cells of the liver towards the M1 phenotype. These cells are resident macrophages of the liver, and this polarization was associated with the pathogenesis of obesity-induced insulin resistance and fatty liver disease, and an increased c-Jun N-terminal protein kinase (JNK1) activation (Naso et al., 2015). Interestingly, removal of Kupffer cells in the liver can improve insulin sensitivity during the consumption of a high-fat diet (Naso et al., 2015). Besides, TNF-$\alpha$ production by M1 macrophages in the liver can promote increased hepatic glucose output via

gluconeogenesis and reduced glycogen content. It can also lead to simultaneous enhanced lipid production and storage by inhibiting intracellular lipases, thereby making fatty acids available for triacylglycerol (TAG) synthesis. Thus, elevated TNF-$\alpha$ in the obese liver may increase blood glucose levels and promote fatty liver disease (Gao et al., 2010).

Conversely, the M2 anti-inflammatory phenotype has significantly reduced pro-inflammatory characteristics, and these cells release high levels of anti-inflammatory cytokines, IL-10. Therefore, maintenance of the M2 phenotype over the M1 phenotype is desirable and essential for appropriate glucose and lipid production and subsequent release. Moreover, amyloid plaques, which characterize islets in T2D, consist mainly of islet amyloid polypeptide (IAPP). Under conditions of chronic hyperglycemia/hyperlipidemia, (pro)IAPP synthesis increases in β-cells, parallel to proinsulin, and reaches threshold levels that allow proapoptotic IAPP oligomers to form (Montane et al., 2012). This induces IL-1β release to recruit macrophages and enhance local islet inflammation (Masters et al., 2010). These data suggest that the high nutrient milieu observed in T2D may activate circulating macrophages that could lead to chronic low-grade inflammation, a hallmark of obesity and T2D. Moreover, interactions of macrophages and the production of pro-inflammatory cytokines can negatively affect metabolic processes in tissues that are physiological targets for insulin. These inflammatory reactions may lead to hyperglycemia and dyslipidemia, which are hallmark characteristics of obesity and T2D.

#### 1.5.2.3.1 Insulin Signalling

Insulin is released into blood by β-cells in response to elevated blood glucose levels following food ingestion. Insulin elicits its anabolic effects via association with the transmembrane insulin receptor (IR) present in target tissues. These key membrane-bound receptors are present in cells that store surplus carbohydrate in the form of glycogen (liver and muscle) or as triacylglycerol (adipose tissue). The IR is a heterotetrameric tyrosine kinase receptor, comprises of four polypeptide subunits (two extracellular $\alpha$-subunits and two transmembrane $\beta$-subunits). The interaction of receptor with insulin induces autophosphorylation of the receptor at tyrosine residues (Tyr1158, Tyr1162, and Tyr1163) (White et al., 1988), initiating the recruitment and phosphorylation of the intracellular adapter proteins (insulin receptor substrate, IRS). Thirteen different IRS isoforms have been described. Of these, isoforms 1 and 2 have been studied extensively since they are widely distributed among different cell types and are mainly activated in skeletal muscle (Corcoran

et al., 2007). Isoforms 1 and 2 are responsible for approximately 75% of insulin-stimulated blood glucose uptake in the body (Corcoran et al., 2007; Shulman et al., 1990). Phosphorylated IRS1 and, to a lesser extent, IRS2 induces activation of the PI3K lipid kinase via binding with the p85 regulatory subunit of PI3K. Activated PI3K converts phosphatidylinositol 3,4-bisphosphate (PIP2) to PI(3,4)P2 and phosphatidylinositol 3,4,5 triphosphate (PIP3) via the p110 catalytic subunit. This conversion activates 3-phosphoinositide-dependent protein kinase 1 (PDK1) that subsequently recruits and phosphorylates protein kinase B (pAkt) at the plasma membrane. PDK1 can also activate atypical protein kinase C (aPKC), regulating glucose metabolism (White, 2003). Downstream of these interactions, pAkt has over 100 substrates that regulate many cellular processes, including cell proliferation, differentiation, endocytosis, survival and glucose homeostasis (Manning and Cantley, 2007). Three isoforms of Akt exist, and Akt2 is most abundant in insulin-sensitive tissues.

Interestingly, when Akt2 was deleted in knockout mice, increased insulin resistance was observed, illustrating the essential physiological role played by Akt2 in mediating glucose homeostasis (Cho et al., 2001). Mechanistically, Akt is an essential regulator of translocation of glucose transported type 4 (GLUT4) vesicles to the plasma membrane, which is critical for the intracellular uptake of free glucose in insulin-sensitive tissues (Henriksen et al., 2011; Taniguchi et al., 2006). Appropriate insulin signalling may be interrupted because of either genetic alterations or physical changes affecting any of the above-mentioned signalling nodes, which may manifest as insulin resistance. Mutations and serine hyperphosphorylation of IRS proteins are mainly associated with development of insulin resistance, as they decrease IRS interaction with PI3K. Araki et al., (1994) have showed that homozygous disruption of IRS1 transcription led to mild insulin resistance, while IRS2-knockout mice exhibited severe insulin resistance (Kubota et al., 2000).

Furthermore, in T2D patients, many precise amino acid substitutions, e.g., Gly972Arg (Mart'ınez-G'omez et al., 2011) in IRS1 proteins, alter protein function but some of these substitutions have been controversial. Hyperphosphorylation of Serine at Ser302, Ser307, Ser612, and Ser632 in IRS1 is responsible for increased insulin resistance (Saini, 2010). Uncontrolled pro-inflammatory cytokine synthesis and secretion and activation of proinflammatory signalling proteins such as TNF-$\alpha$ and the isoform 1 of JNK1 are responsible for fatty tissue expansion, which can induce serine hyperphosphorylation in IRS1 (Hotamisligil et al., 1996; Stuart et al., 2014), especially at residue Ser636. However, it is not

known which specific serine residues, or a combination require hyperphosphorylation to elicit the insulin-resistant phenotype, as excessive phosphorylation at Ser337 and Ser636 has been demonstrated in muscle samples from patients with metabolic syndrome, but not at Ser307, Ser789. or Ser1101 as reported by others (Stuart et al., 2014). Moreover, serine hyperphosphorylation at residue Ser312 marks IRS1/2 for degradation, which dampens the IR-mediated signalling relay. Taken together, these data not only demonstrate the complexity of the role played by IRS proteins but also their importance in modulating insulin resistance. The role of inflammation in disrupted insulin signalling and insulin resistance is shown in Fig. 1.5.

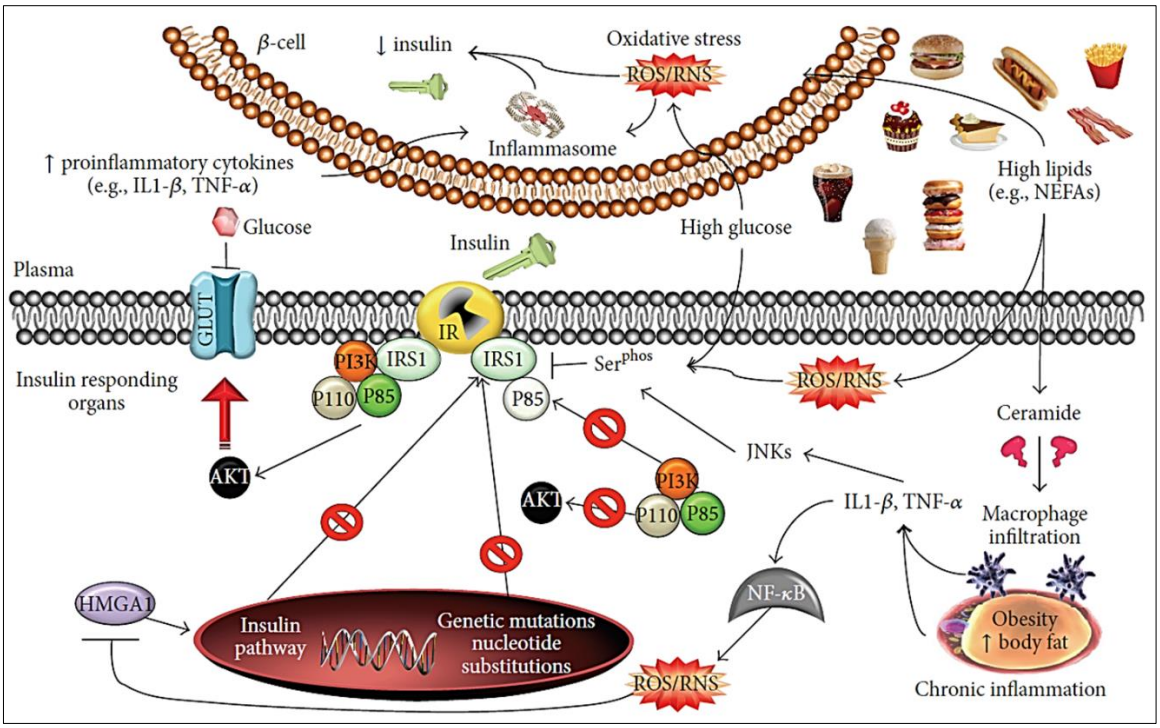


**Figure 1.5 Role of inflammation in insulin resistance**. Overnutrition leads to high levels of lipids and glucose and, overtime development of obesity and metabolic syndrome (MetS), ultimately causing chronic low-grade inflammation. High nutrients can modulate insulin resistance by altering the insulin-signalling cascade through IRS1, PI3K, and AKT phosphorylation changes. High lipids can also promote inflammation through ceramide generation, and increased glucose increases overall oxidative stress. During T2D progression, the insulin resistant tissues promote the exhaustion of insulin secreting β-cells, which activates defensive mechanisms leading to lower insulin release (Keane et al., 2015).

#### 1.5.2.3.2 Tumour Necrosis Factor Alpha (TNF-α)

TNF-α is a potent immunoregulatory cytokine produced by many cells, including adipocytes, keratinocytes, mast cells, Langerhans cells, monocytes and macrophages. It is implicated in the pathogenesis of a wide range of human diseases, including diabetes (Chen et al., 2002). Hotamisligil et al., (1993) have reported TNF-α to be the first proinflammatory cytokine

associated with obesity and related insulin resistance. TNF-α can activate the mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-kB) signalling pathways, resulting in the release of other pro-inflammatory cytokines such as IL-1β and IL-6 (McArdle et al., 2013). Yuan et al., (2001) identified the IKKB pathway as a target for TNF-α induced insulin resistance. Kern et al., (1995) showed that obese individuals have 2.5-fold more TNF-α in their adipose tissue than lean controls. Hotamisligil et al., (1994) demonstrated that TNF-α participates in obesity-related systemic insulin resistance by inhibiting insulin receptor tyrosine kinase activity in skeletal muscle and adipose tissues. Peraldi et al., (1996) also reported that recombinant human TNF-α inhibits the insulin-dependent tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate 1 (IRS-1) in adipocytes and myeloid 32D cells. Later, studies suggested that the defect in insulin signalling could be attributed to serine phosphorylation of IRS1 at serine-307 residue by activation of JNK1, providing the first explanation relating inflammation and insulin resistance (Aguirre et al., 2000; Hotamisligil et al., 1996). At a molecular level, stimulation of cells with TNF-α or increased levels of free fatty acids inhibited phosphorylation of serine residues of IRS1 (Aguirre et al., 2000). Also, treatment of 3T3-L1 adipocytes with TNF-α resulted in reduced GLUT4 protein levels along with decreased activity of protein kinase B (Akt) (Ruan et al., 2002). Apart from inhibiting the insulin signalling pathway, TNF-α also impairs insulin secretion (Tsiotra et al., 2001). Neutralization of TNF-α led to improved insulin sensitivity in animal models and human subjects (Kern et al., 1995; Uysal et al., 1997). Taken together, these data demonstrated that TNF-α is a key mediator of insulin resistance in obesity, and neutralizing it might ameliorate obesity-induced insulin resistance in T2D patients. Furthermore, in humans, the gene for TNF-α maps to chromosome 6p21.3 and eight SNPs (−1031T/C, −863C/A, −857C/T, −575G/A, −376G/A, −308G/A, −244G/A, and −238G/A) have been identified within the TNF-α promoter (Bayley et al., 2004). One of the promoter SNPs in the TNF-α gene (-308 G/A) showed a two-fold increase in *TNF-α* transcript levels (Guzmán-Flores et al., 2011; Kroeger et al., 1997) and altered circulating FFAs in obese T2D patients (Fontaine-Bisson et al., 2007). Several studies showed association of TNF-α promoter SNPs with T2D (Banerjee et al., 2014)

#### 1.5.2.4 GPCRs in β-Cell Dysfunction and Insulin Resistance

Currently, there are more than 30 G protein-coupled receptors (GPCRs) that have been implicated in the development and progression of β-cell dysfunction, insulin resistance, obesity and T2D, as shown in Fig. 1.6 (Riddy et al., 2018).

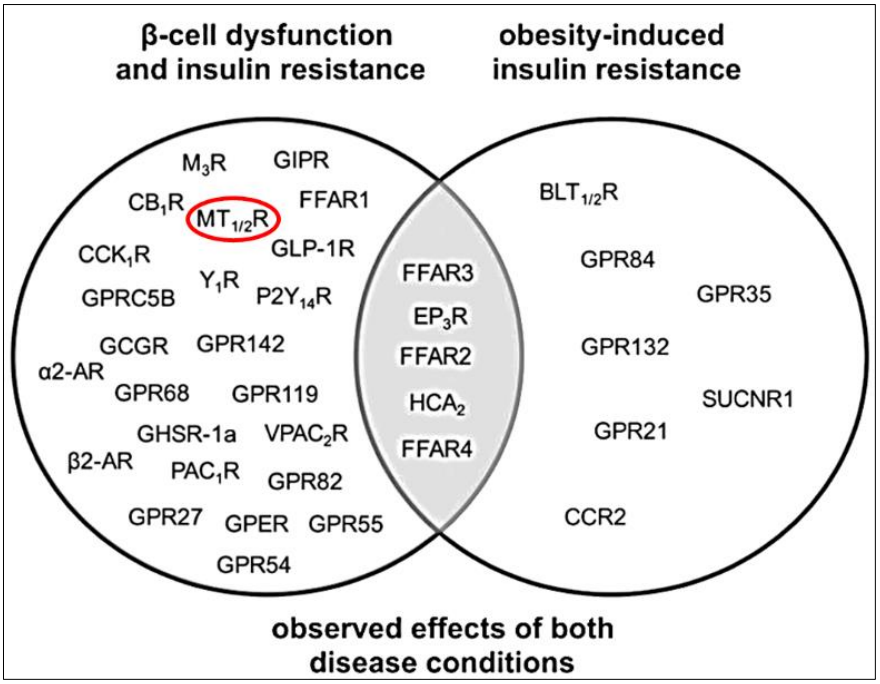


**Figure 1.6 Involvement of GPCRs in the development and/or progression of β-cell dysfunction, insulin resistance and obesity-induced T2D.** (Riddy et al., 2018)

#### 1.5.2.4.1 Melatonin Receptors in β-cell Dysfunction and Insulin Resistance

The pineal gland primarily secretes melatonin (MT) during the dark phase. Melatonin is also secreted to a lesser extent from the innate immune system, the gastrointestinal tract and the retina. The effects of MT are mediated by two homologous but distinct GPCRs, namely MT1 and MT2 receptors (MTRs), encoded by two separate genes, MTNR1A and MTNR1B, respectively. MTRs are Gαi coupled and expressed in a cell-specific manner in mice and humans. MTRs are involved in numerous physiological and neuroendocrine functions. In humans, MTNR1B locates on chromosome 11q14.3. Interest in MT in the pathogenesis of T2D have mainly come from three independent genome-wide association studies that led to the identification of several polymorphisms located near the *MTNR1B* gene. These were associated with increased fasting blood glucose, a reduction of early insulin response to glucose, and ultimately an increased risk of developing T2D (Bouatia-Naji et al., 2009; Lyssenko et al., 2009). This genetic association is robust and subsequently replicated by several groups in other populations (Renström et al., 2015; Ronn et al., 2009). However, the influence of MT on insulin secretion is still debated, with conflicting results generated *in vitro* using rodent and human islets (Costes et al., 2015; Lyssenko et al., 2009). Furthermore, MTRs are expressed at low levels in both α- and β-cells. Tuomi et al., (2016) have demonstrated that the common rs10830963 variant located in the MTNR1B intron is an

expression quantitative trait locus conferring increased expression (two- to four- fold) of the receptor in islets, as measured by RNA Seq in 204 Scandinavian donors. Furthermore, studies suggest that increased MT signalling is a risk factor for T2DM. However, analysis of rare loss-of-function MTNR1B variants suggested that reduced MT signalling increases T2D risk (Bonnefond et al., 2012; Prokopenko et al., 2009). Lane et al., (2016) have reported that the *MTNR1B* rs10830963 G (risk) allele influences MT secretion dynamics with consequences on the sleep/wake cycle, and sleep disturbances are implicated in the dysregulation of blood glucose levels and increased T2D risk (Tsuneki et al., 2016).

## 1.6 Diabetes Management

Diabetes, if left untreated or undertreated, can cause many microvascular complications in the body, leading to poor quality of life and reduced lifespan. Possible complications that are related to DM are cardiomyopathy, neuropathy, nephropathy and retinopathy. Glucose regulation during the early stages can decrease the possible progression towards multisystem complications of microvascular and macrovascular endpoints (Forbes and Cooper, 2013). There have been many advancements made in the past decade for the treatment of T1D and T2D.

### 1.6.1 Type 1 Diabetes Management

T1D is a chronic disease that eventually leads to complete loss of insulin due to the destruction of β-cells. Also, the lack of appropriate islet cell repair mechanisms ultimately affects glycemic control. As a result, insulin replacement therapy is currently the first-line therapeutic option for treating T1D (Pathak et al., 2019). The current insulin centric therapeutic approach renders a T1D patient susceptible to severe hypoglycaemia episodes, lifelong dependency on exogenous insulin, insulin resistance, mild obesity, and psychiatric conditions (Jacobson et al., 2013; Priya and Kalra, 2018; Yeh et al., 2012). Despite of successful implementation of multiple insulin delivery devices, maintaining normoglycemia without frequent hypoglycemic episodes remains a considerable challenge for health care providers. As a result, the clinical practice has gradually moved towards using continuous insulin infusion systems for insulin delivery which enables greater control over HbA1c and lower incidences of hypoglycemia.

Along with insulin therapy, many other interventions are being currently used in clinical trials such as artificial pancreas, immune modulation, sodium-glucose co-transporter-2 (SGLT2)

inhibitor, β-cell encapsulation, microencapsulation, stem cell therapies, incretin therapies, dipeptidyl peptidase- IV inhibitors (DPP-IV) and islet transplantation (DeGeeter et al., 2016; Pathak et al., 2019; Wang et al., 2018). Although the recent emergence of fast-acting and long-acting insulin analogues has improved the quality of life for T1D patients, many challenges remain. Transplantation of primary islets offers exciting prospects for treating T1D patients. However, islets limited availability is one of the obstacles for widespread use of islets transplantation. To overcome the shortage of donors, xenotransplantation of islets has been explored, but ethical considerations and concerns about transgenic islets genetic stability remain. Therefore, in the current situation, insulin replacement therapy and transplantation of islets from humans remain a practical and financially feasible option to treat T1D. The development of effective human islet transplantation strategies is also hampered due to extensive death of islet cells during the immediate period post-transplantation. This inevitably increases the requirement for the number of islets needed to achieve glycemic control and insulin independence. Besides, disruption of typical islet architecture and morphology and poor vascular engraftment during the post-transplantation period also significantly contribute to the deterioration of islet graft function (Harlan et al., 2009; Pathak et al., 2019). New approaches are needed for successful therapeutic outcomes and complete insulin independence. In this direction, conventional T1D therapeutic methods including insulin replacement, SGLT2 inhibitors, immune therapies, DPP-IV inhibitors and peptide agonists, need to be considered in combination with emerging approaches for optimum clinical therapeutic outcomes.

### 1.6.2 Type 2 Diabetes Management

To manage T2D, a combination of lifestyle changes and pharmacological treatment is necessary. At present, different treatments, both oral and injectable are available for the management of T2D. Metformin remains the first choice of treatment for most patients. Another alternative or second-line treatment is individualized depending on the characteristics of each patient. Dietary intake and physical exercise are the two main determinants of energy balance. A good amount of sleep (~7 hrs) is considered fundamental in treating T2D patients (Garber et al., 2016). Sleep deprivation aggravates insulin resistance, hypertension, hyperglycemia, and dyslipidemia (McNeil et al., 2013). Further, oral drugs are used for the management of T2D, such as sulfonylureas (metformin), alpha-glucosidase inhibitors (acarbose, miglitol and voglibose), thiazolidinediones (rosiglitazone and pioglitazone), DPP-IV inhibitors (sitagliptin, saxagliptin, linagliptin, alogliptin, vildagliptin),

and SGLT2 inhibitor (dapagliflozin, canagliflozin, empagliflozin). Injectable drugs include glucagon-like peptide 1 receptor agonists (GLP-1 RA) (exenatide, lixisenatide, liraglutide, albiglutide, dulaglutide) and insulin. These treatment modalities work on different organs to regulate blood glucose levels. Biguanides and thiazolidinediones reduce glucose production in the liver and increase insulin sensitivity in the skeletal muscle and adipose tissue. SGLT2 inhibitors reduce glucose reabsorption in the kidney. Sulfonylureas and Meglitinides increase insulin secretion in the pancreas. GLP-1 RA and DPP-IV inhibitors increase insulin secretion and decrease glucagon secretion, along with amylin and alpha-glucosidase inhibitors, they also slow gastric emptying while increasing satiety (Marín-Peñalver et al., 2016; Pramanik et al., 2018). Despite the several treatment options available for the management of T2D, most of the patients do not achieve normoglycemia and suffer from major or minor side effects such as hypoglycemia, weight loss, stomach upset, tiredness, liver diseases, kidney complications etc. As the existing therapies only help alleviate hyperglycemia and other symptomatic characteristics, recent research is directed to develop novel drugs with minimal side effects. Though a total cure is still elusive, gaining insights into the possible modes of β-cell preservation and regeneration-based therapies are crucial for the management of both T1D and T2D.

## 1.7 β-Cell Regeneration

The ultimate goal of β-cell regeneration research is to expand endogenous β-cell mass without compromising function to prevent or treat diabetes. β-cell regeneration is achieved broadly in three different ways i.e., β-cell proliferation, β-cell neogenesis and β-cell transdifferentiation.

### 1.7.1 β-Cell Proliferation

Self-renewal of existing β-cells is an attractive approach for generating new β-cells. β-cells usually proliferate in the developing (embryonic and neonatal) mouse and human pancreas and can be stimulated to replicate by several metabolic stressors, including pregnancy and obesity (Baeyens et al., 2016; Cox et al., 2016). During the early postnatal period, proliferation is the primary mechanism of β-cell expansion to generate sufficient β-cell mass (Meier et al., 2008). However, β-cell proliferation rapidly declines early in life, and in adults, the rate of β-cell division is meagre. To date, the identification of molecules that can activate replication in adult β-cells has proven challenging, partly due to species-dependent molecular differences between mouse and human β-cells. Factors that can stimulate replication of

mouse β-cells do not necessarily induce expansion of human β-cells (Aamodt et al., 2016; Karakose et al., 2018). Another impediment is that adult β-cells are refractory to mitogens that stimulate proliferation in juvenile β-cells from younger donors (Dai et al., 2017). Unlike juvenile β-cells, adult β-cells have increased expression of cell cycle inhibitors such as p16INK4a and a reduction in cell cycle activators including FoxM1, cyclins, and cyclin-dependent kinases that render them resistant to proliferation (Fiaschi-Taesch et al., 2013; Golson et al., 2015; Tschen et al., 2017).

Furthermore, it is hypothesized that adult β-cells are resistant to rapid turnover to prevent hyperinsulinemia. Thus, one valid concern is that inducing unrestrained β-cell growth in people could lead to the formation of insulinomas and potential lethality due to hypoglycemia. The recent insight into β-cell heterogeneity further complicates the search for factors that can activate replication of adult β-cells. While it is clear that different subpopulations of β-cells exist within an islet, it is not known whether all β-cells can proliferate. Flattop (Fltp), an effector of Wnt/planar cell polarity signalling, was shown to mark a population of mature β-cells with greater functionality but lower the proliferative potential, suggesting that there may be a subset of β-cells that have a more remarkable ability to proliferate than others (Bader et al., 2016; Dorrell et al., 2016). Identifying markers delineating cells with a higher proliferative capacity would potentially allow researchers to target this population specifically. However, β-cell proliferation often appears to occur at the expense of insulin secretion, and replicating β-cells tend to resemble immature β-cells more closely. For example, when replication in adult mouse β-cells was induced by exogenously expressing c-Myc, these β-cells displayed reduced expression of genes essential for glucose sensing and insulin secretion (GLUT2, PC1/3), as well as transcriptional markers of mature β-cells (PDX1, MAFA, NKX2.2) (Puri et al., 2018). Thus, the balance between proliferation and functionality must also be considered when identifying new drugs to expand β-cell mass.

### 1.7.2 β-Cell Neogenesis

Pancreatic β-cells are initially formed during embryonic development from an endocrine progenitor population that lies within the pancreatic ductal epithelium and is marked by the transcription factor Neurogenin3 (NGN3). In mice and humans, $NGN3^{+}$ endocrine progenitor cells differentiate into all four adult endocrine cell types during embryogenesis, but a decline in numbers occurs upon birth (Gradwohl et al., 2000; Gu et al., 2002). Because endocrine cells originate from the ductal epithelium during development, many researchers have

examined whether the embryonic endocrine differentiation program can be reactivated in adult pancreatic ducts to serve as a potential source of new β-cells. However, whether this occurs endogenously or under certain pathological conditions remains controversial. Several studies using pancreatic injury models, such as pancreatic duct ligation or partial pancreatectomy, have shown the reappearance of $NGN3^+$ progenitor cells within the adult ductal epithelium and the presence of tiny clusters of endocrine cells close to these ducts, suggesting the occurrence of neogenesis (Ackermann Misfeldt et al., 2008; Xu et al., 2008; Van de Casteele et al., 2013). However, studies using similar approaches provide evidence that neogenesis does not occur, suggesting this mechanism to be difficult to activate or relatively rare (Cavelti-Weder et al.; 2013; Menge et al., 2008; Ranking et al., 2013). Lineage tracing of the ductal tree using an inducible Cre recombinase (CreER) driven by a fragment of the human carbonic anhydrase promoter provided evidence that mature ducts can give rise to endocrine cells, whereas experiments using Hnf1CreER and Sox9CreER showed evidence to the contrary (Inada et al., 2008; Kopp et al., 2011). In humans, obtaining proof of β-cell neogenesis has also been challenging. Without the ability to perform genetic lineage tracing of human ductal cells, it is difficult to confirm that human β-cell neogenesis appreciably occurs *in vivo*.

### 1.7.3 β-Cell Transdifferentiation

While it remains unclear whether, under which conditions ductal cells can be reactivated to differentiate into β-cells, there is mounting evidence to suggest that other differentiated tissue types can be reprogrammed into β-cells in a process referred to as transdifferentiation. During embryonic development, the pancreas forms from a foregut endoderm region marked by pancreatic and duodenal homeobox factor 1 (PDX1) expression, which is posterior to the antral stomach, adjacent to the budding liver and anterior to the duodenum (Guney et al., 2009). Due to their common developmental lineage, it can be speculated that cells from these closely related endodermal organs could be reprogrammed into pancreatic endocrine cells. Indeed, several studies have demonstrated that insulin-positive cells can be induced *in vivo* in other tissues like liver, kidney and gut by the adenoviral transduction of one or a combination of pancreatic transcription factors, including PDX1, neuronal differentiation 1 (NeuroD1), or a combination of PDX1, NGN3, and MAFA (known as the PNM factors) (Banga et al., 2012; Ferber et al., 2000; Kojima et al., 2003). Within the pancreas itself, terminally differentiated exocrine tissue has also been suggested as a *de novo* endocrine cell source. In 2008, Zhou et al., found that adenoviral delivery of the PNM factors into the pancreas of an adult immune-

compromised mouse could convert acinar cells into insulin-producing β-cells. However, pancreatic exocrine tissue's intrinsic capacity to generate β-cells without adenoviral administration is being unravelled (Clayton et al., 2016; Desai et al., 2007).

Furthermore, recent attention has also been focused on understanding whether other endocrine cell types within the islet have the regenerative potential to convert into β-cells. Although endocrine cells which were thought to be stable, terminally differentiated population, studies have shown that they exhibit considerable plasticity under stress conditions or genetic manipulation (Gutierrez et al., 2017; Swisa et al., 2017). In an adult mouse model with extreme β-cell loss and hyperglycemia, α-cells coexpressed insulin, and some of these bihormonal cells were shown through genetic lineage tracing to become monohormonal insulin-positive cells over time (Thorel et al., 2010). Subsequent studies have shown that reprogramming can occur throughout the mouse's lifetime in response to physiological stimuli such as multiple rounds of pregnancy, and also δ-cells can convert to β-cells in young mice after β-cell injury (Chera et al., 2014; Ye et al., 2016). Reprogramming of α-cells to β-cells has also recently been suggested to occur usually without stimuli or injury. A population of immature β-cells identified by the presence of insulin expression, but the absence of the maturity marker urocortin3 (Ucn3) was found at the periphery of the islet and are thought to be in a transition state between mature cells and β-cells (van der Meulen et al., 2017). Due to the inability to perform lineage tracing experiments in humans, the question still remains whether endocrine cells can transdifferentiate into β-cells in patients with pancreatic diseases.

Thus, the process of endocrine transdifferentiation in humans or mice requires dedifferentiation before reprogramming has not yet been answered definitively, as both direct reprogramming and transdifferentiation associated with dedifferentiation (with or without re-expression of NGN3) have been reported (Chakravarthy et al., 2017; Talchai et al., 2012). Studies on ectopic expression of the transcription factor PAX4 and inhibition of the α-cell gene ARX in mice found that α-cells get converted to β-cells (Collambat et al., 2009; Courtnet al., 2013). Recently, it was reported that the deletion of the DNA methyltransferase, Dnmt1, and ARX is necessary for converting α-cells to functional β-cells (Chakravarthy et al., 2017). The expression of PDX1 and MAFA specifically in α-cells using a genetic approach or throughout the pancreas using a viral approach can also induce insulin expression in α-cells, and interestingly viral approach could rescue blood glucose levels in the non-obese diabetic (NOD) model of autoimmune diabetes (Matsuoka et al., 2017; Xiao et

al., 2018). In addition, studies have also reported β-cell regeneration *in-vitro* through transdifferentiation of human pluripotent stem cells (hPSCs) (Rosler et al., 2004) and induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006) which are pluripotent non-pancreatic progenitors. This newly formed β-cells when transplanted into diabetic mice and rats, were able to secrete insulin (Kroon et al., 2008; Rezania et al., 2014; Velazco-Cruz et al., 2019). Despite many recent scientific advances, it remains uncertain whether human pancreatic β-cells possess an intrinsic regenerative capacity. However, available data suggest that β-cells could exhibit the potential for regeneration under favourable conditions. Different approaches for β-cell regeneration and the pancreatic lineages are shown in Fig. 1.7.

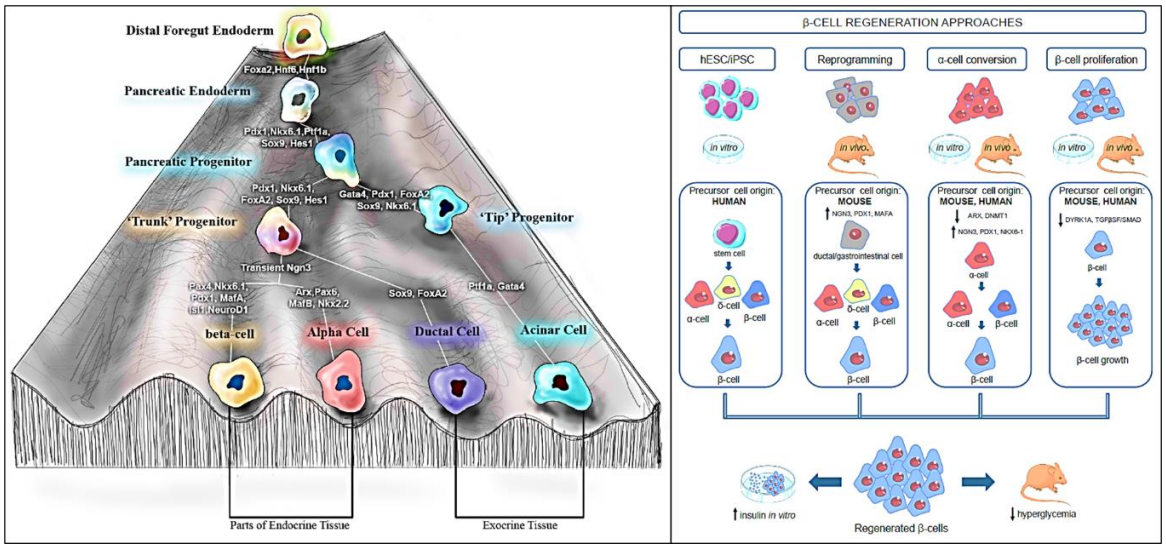


**Figure 1.7 Differentiation of pancreatic lineages and strategies for β-cell regeneration.** Novel β-cells can be derived through transdifferentiation of human pluripotent stem cells (hPSC) and induced pluripotent stem cells (iPSC), reprogramming of mature, non-endocrine (ductal and gastrointestinal) cell populations, α- to β-cell conversion and β-cell proliferation (Nasteska et al., 2019; Singh and Ninov, 2018).

### 1.7.4 Potent β-cell Regenerative Drugs

Candidate approaches to β-cell regenerative drug discovery have suggested that gamma-aminobutyric acid (GABA), dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) inhibitors, GLP-1, prolactin/placental lactogen (PRL/PL), osteoprotegerin/denosumab, inhibitors of the receptor activator of nuclear kappa-B ligand (RANKL), the TGF-β superfamily, serpin B1, cell cycle inhibitors (p18, p21) and a V-growth factor (VGF)-derived peptide called TLQP-21 may have mitogenic effects on β-cells. (Karakose et al., 2018; Rathwa et al., 2020).

## 1.8 Melatonin: Hormone of Darkness with Pleiotropic Effects

Changing lifestyle trends such as a tendency to nocturnality and excessively calorie-rich diets cause disturbance of the sleep/wake cycle and other circadian rhythms (Bixler, 2009). Deviation in circadian patterns favours the occurrence of diabetes (Scheer et al., 2009). Inconsistent data have been reported concerning the pineal hormone's effect on insulin secretion, blood glucose, and carbohydrate metabolism. Melatonin (N-acetyl-5-methoxytryptamine) a tryptophan derived small indolic molecule, is mainly secreted by the pineal gland and locally in several other tissues (Reiter et al., 1991; Stefilj et al., 2001). Melatonin is known as the hormone of darkness, circadian rhythm regulator, and has pleiotropic effects. In mammals, the concentration of plasma melatonin during the night is found to be 80–100 pg/mL and low during the day (10–20 pg/mL) (Simonneaux and Ribelayga, 2003). Its synthesis comprises of two steps, initially the conversion of amino acid tryptophan into serotonin (5-hydroxytryptamine, 5-HT), further acetylation by arylalkylamine N-acetyltransferase (AA-NAT), the rate-limiting step in melatonin biosynthesis, before finally being converted into melatonin by hydroxyindole- O-methyltransferase (HIOMT) (Axelrod and Weissbach, 1960). In diabetic patients, a reduction in melatonin levels and insulin was observed. On this basis, melatonin may perhaps be involved in the genesis of diabetes, and a functional inter-relationship between melatonin and insulin was observed (Peschke et al., 2006). Melatonin mediates its action by two receptors MT1 and MT2, as discussed before. These receptors are found in the pancreas (α, β, and δ cells) and insulin-sensitive tissues apart from several other tissues. The intracellular signal transduction pathways of the pancreatic β-cell influenced by melatonin via MT1- and MT2-membrane receptors include cAMP-, cGMP IP3-signaling pathways shown in Fig. 1.8. Melatonin inhibits cAMP and cGMP stimulated insulin secretion mediated via Gi protein-coupled MT1 and MT2 receptors. Alternatively, melatonin induces IP3 liberation that allows $Ca^{2+}$ to flow into the cell from intracellular stores (Bach et al., 2005), a standard mechanism that triggers insulin secretion by pancreatic β-cell. The MT2r receptor-dependent signalling pathway of melatonin stimulates phospholipase C via Gq proteins, markedly elevating inositol triphosphate (IP3)/$Ca^{2+}$ from intracellular stores (Peschke et al., 2006). The co-product of phospholipase C (PLC) activity, diacylglycerol (DAG), may lead to MAPK p38-modulated activation of protein kinase D (PKD), protein kinase C (PKC) and increased insulin vesicle fusion. In humans, melatonin administration reduced glucose tolerance by decreasing insulin release in the morning, while a decline in insulin sensitivity was observed in the evening (Rubio-Sastre et al., 2014). Furthermore, in mouse liver, melatonin is required for insulin-

stimulated phosphatidylinositol 3-kinase (PI3K)–AKT activity, in rats, it suppresses hepatic glucose production, and in the human hepatocyte cell line HepG2, it activates glycogen synthesis, probably via a PKCζ–AKT–glycogen synthase kinase-3β (GSK3β) pathway. In mouse skeletal muscle, melatonin activates the insulin receptor substrate 1 (IRS1)–PI3K–PKCζ pathway to enhance glucose uptake rate. In inguinal rat adipocytes, melatonin inhibits the cAMP–PKA pathway and isoproterenol-induced lipolysis and fatty acid transport in some cases. In the human brown adipocyte PAZ6 cell line, melatonin acutely inhibits cGMP production and decreases glucose transporter type 4 (GLUT4) expression and glucose uptake upon long-term treatment (Karamitri and Jockers, 2019). Melatonin is a natural, free radical scavenger that modulates Nrf2 (Ahmadi and Ashrafizadeh, 2020) and has anti-inflammatory, antioxidant effects (Hacışevki and Baba, 2018). Different animal studies suggest that melatonin supplementation may benefit glucose homeostasis and body weight regulation under certain circumstances. DM is more prevalent in middle-aged and older adults (40-60 years). The fact that melatonin declines with age should encourage more preclinical trials in various experimental diabetes models, leading to clinical trials in humans to evaluate melatonin's therapeutic potential in diabetes.

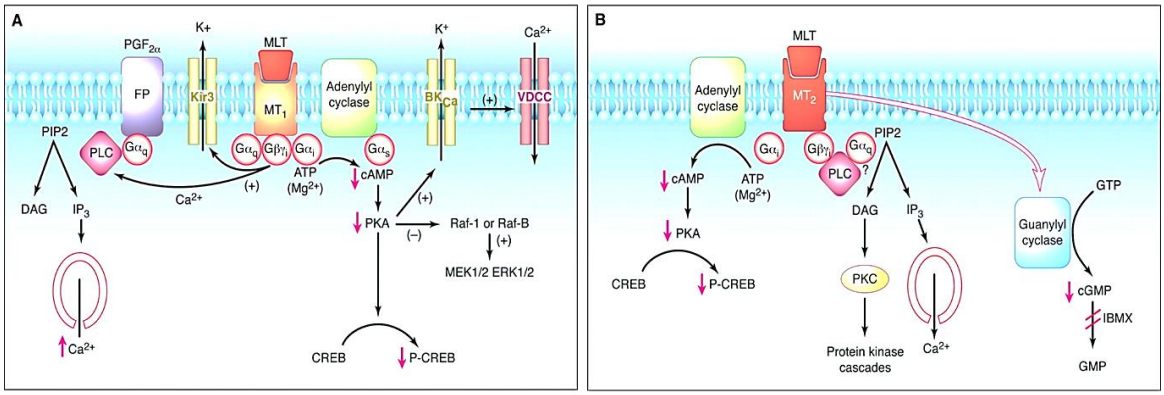


**Figure 1.8 Putative signalling pathways activated by MT1 and MT2 melatonin receptors.** (A) Multiple signalling pathways for MT1 melatonin receptors coupled to Gαi and Gαq/11. (B) Signalling pathways coupled to MT2 melatonin receptor activation. No direct evidence for MT2 receptors coupling to Gq has been reported, so the pathway leading to PKC activation remains putative. PIP2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; DAG, diacylglycerol; PKA, protein kinase A; CREB, cAMP-responsive element-binding protein; ER, endoplasmic reticulum; VDCC, voltage-dependent Ca2+ channel; BKCa, calcium-activated potassium channel; FP, receptor for prostaglandin F2α; PGF2α, prostaglandin F2α; IBMX, isobutylmethylxanthine; ATP, adenosine triphosphate; MLT, melatonin; GTP, guanosine triphosphate; GMP, guanosine monophosphate (Masana and Dubocovich, 2001).

## 1.9 Incretins and DPP-IV Inhibitors

The incretin effect is diminished in T2D (Gallwitz, 2007). Hence, among the currently available antidiabetic drugs, the incretin-based therapies, GLP-1 RAs, and DPP-IV inhibitors

are widely used antidiabetic drugs that target β-cell function and mass, as well as other systems that contribute to T2D (Fig. 1.9). Two gut hormones, gastric inhibitory polypeptide (GIP; now referred to as glucose-dependent insulinotropic polypeptide) and GLP-1, are endogenous incretins that potentiate GSIS (Yabe and Seino, 2011). GIP is a 42-amino-acid hormone secreted from K cells in the upper small intestine, and GLP-1 is a 31-amino-acid hormone produced from a proglucagon precursor secreted from L cells in the lower intestine and colon. GLP-1 and GIP account for 50% to 70% of postprandial insulin secretion from pancreatic β-cells. Once secreted, GLP-1 and GIP are rapidly degraded by the ubiquitous enzyme DPP-IV, which inactivates incretins by cleaving their 2 N-terminal residues. Incretin-bound receptors increase intracellular cAMP levels, thereby activating protein kinase A (PKA) (Fehmann et al., 1995) and cAMP-activated guanine nucleotide exchange factors that target Ras-like GTPases 2 (Epac2; also referred to as cAMP-GEF-II) (Holz, 2004). PKA and Epac2 mediate ion-channel activity changes and enhance cytosolic calcium levels and the exocytosis of insulin-containing granules. Together, these events contribute to insulin secretion stimulation in a glucose-dependent manner (Yabe and Seino, 2011). Both GIP and GLP-1 have demonstrated non-insulinotropic actions, such as controlling β-cell proliferation and survival (Yabe and Seino, 2011; Dalle et al., 2013). GIP has also exhibited an anti-apoptotic function in pancreatic β-cells that is mediated by the activation of the cAMP response element-binding protein (CREB) and protein kinase B (Akt/PKB) pathways. By activating the cAMP/PKA/CREB, PI3K, and ERK1/2 pathways, GLP-1 is considered a growth and differentiation factor for mature β-cells and β-cell progenitors. GIP and GLP-1 induce the transcription of cyclin D1, a molecule critical for cell cycle progression from the first gap phase (G1) to the synthesis (S) phase in most cell types. GLP-1 also replenishes insulin stores and prevents β-cell exhaustion by upregulating insulin at the mRNA and protein levels via PKA-dependent and -independent signalling pathways. GIP activates the Raf-Mek1/2-ERK1/2 signalling pathway via cAMP/PKA signalling in GIPR, which induces β-cell proliferation (Dalle et al., 2013).

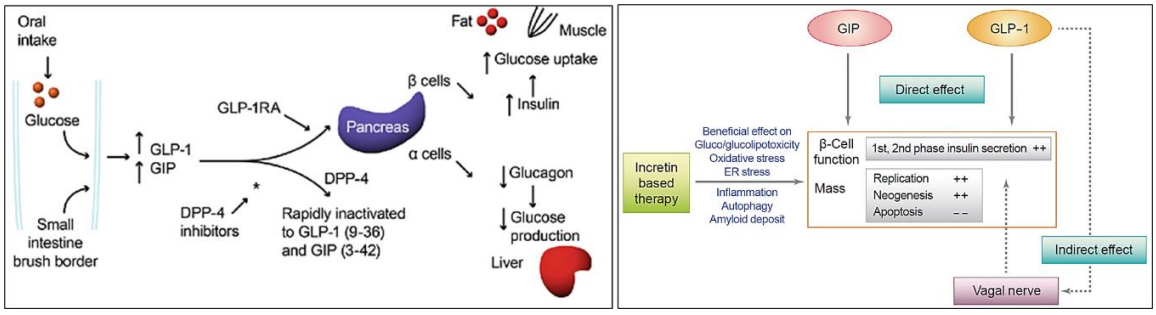

**Figure 1.9 Mechanism of action of DPP-IV inhibitors and GLP-1RAs and its effect on pancreatic β-cell in animal models.** GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; ER, endoplasmic reticulum (Chon et al., 2014; Tibaldi, 2014).

DPP-IV inhibitors are orally administered small molecule drugs that compete with DPP-IV substrates for the active sites in the enzyme and inhibit >80% of DPP-IV activity. Thus, DPP-IV inhibitors increase circulating levels of endogenous active GLP-1 and GIP by approximately 2 to 3-fold and exert a glucose-dependent dual action on both α- and β-cell function that stimulates insulin secretion and suppresses glucagon secretion under hyperglycemic conditions (Chon and Gautier, 2016). Currently, multiple DPP-IV inhibitors are approved and available for their use in the treatment of T2D, including sitagliptin, vildagliptin, saxagliptin, linagliptin, anagliptin, alogliptin, gemigliptin. In addition, several other DPP-IV inhibitors are pending approval. The various DPP-IV inhibitors slightly differ in their structure, absorption rate, distribution, metabolism, and elimination and their potency and duration of action (He et al., 2015). DPP-IV inhibition exerts long-lasting effects on pancreatic islet mass and/or insulin content, an influence not observable with sulfonylurea (Matveyenko et al., 2009). Further, a report suggests that DPP-IV inhibitors might promote transplanted stem cells' differentiation and play an immunoregulatory role in autoimmune insulitis (Kim et al., 2009). From the above- mentioned drugs, sitagliptin is a highly selective DPP-IV inhibitor that has been approved for T2D therapy. Sitagliptin is effective, well-tolerated, and safe for treating T2D in monotherapy or in combination therapy with metformin or thiazolidinediones. It reduces the glycemic parameters HbA1c and fasting and postprandial glucose and improves β-cell function. Sitagliptin is weight neutral and does not increase the incidence of hypoglycemic episodes or other adverse events, and is superior in reducing HbA1c than other oral anti-hyperglycemic drugs (Gallwitz, 2007). The effect of DPP-IV inhibitors on β-cell mass in humans remains unevaluated in clinical studies. In case the observed effects on β-cell function and mass in preclinical studies also apply to human studies, sitagliptin could also have the potential to be useful in pre-diabetic stages to prevent the progression of diabetes. Although a growing body of evidence suggests that incretin-based therapies can modify the natural course of diabetes, further studies are needed to evaluate the long-term effects of incretin-based therapy on β-cell function and mass and glucose control.

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