Chapter 1
Introduction and Review of Literature

1. Introduction

The rapid development of humankind has led to progress, but it has also been enduring numerous lifestyle diseases. Emerging technologies have significantly altered people's daily routines, resulting in multiple lifestyle disorders such as diabetes, metabolic syndrome, dyslipidemia, overweight/obesity, hypertension, and cardiovascular disease. In recent years, the incidence of these diseases has risen alarmingly, particularly among Indians, due to the rapid economic growth and shift towards a more Westernized lifestyle over the past few decades (Pappachan, 2011; Oberoi and Kansra, 2020).

1.1 Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from defective insulin secretion and/or increased cellular resistance to insulin. The distinction between DM is based on age at onset, degree of loss of β-cell function, degree of insulin resistance, presence of diabetes-associated autoantibodies, and requirement for insulin therapy for survival (Leslie et al., 2016). Persistently high blood sugar levels in individuals with DM can result in serious macrovascular issues such as ischemic heart disease, stroke, and peripheral artery diseases, as well as microvascular complications such as neuropathy, retinopathy, and nephropathy (Chawla et al., 2016).

1.2 Classification

DM is classified into three main categories: type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes (GDM).

a) T1D (juvenile-onset diabetes/insulin-dependent diabetes) is characterized by autoimmune β-cell destruction, which results in absolute insulin deficiency. It accounts for 5-10% of the total number of diabetic cases. Regular injection of insulin or its analogues is necessary for the therapy (ADA, 2023). The precise etiology of T1D is unknown. However, T1D develops in early life due to a complex interplay of environmental and genetic factors (WHO, 2016).
b) T2D (adult-onset diabetes/non-insulin dependent diabetes) is a metabolic disorder manifested by insulin resistance in peripheral tissues due to impaired insulin signaling cascade. The pathogenesis of T2D is more complex, comprising different degrees of β-cell failure and insulin resistance (Kahn, 2003). T2D accounts for 95% of the total number of diabetic cases. The fundamental causes of T2D include ethnicity, family history, obesity,

unhealthy dietary pattern, and a lack of physical activity (WHO, 2016). The boundary line between normoglycemia and diabetes is frequently defined by "Impaired Glucose Tolerance" (IGT) or "Impaired Fasting Glycemia" (IFG). Prediabetes is the term used to describe persons with IGT/IFG (IDF Diabetes Atlas, 2021), and it is expected that one in every four people with IGT/IFG will develop T2D during the next 3-5 years (IDF Diabetes Atlas, 2021).

c) High blood glucose levels define GDM during pregnancy in women without a prior DM history (ADA, 2023). GDM increases the risk of developing T2D for both mother and offspring later in life. Furthermore, GDM has been associated with the development of cardiovascular disease and metabolic syndrome (Coustan, 2013). During pregnancy, the placenta produces a variety of hormones to aid to the development of the fetus, some of which can interfere with insulin action. The hormones such as cortisol and estrogen have strong diabetogenic effects (Coustan, 2013), gradually leading to insulin resistance.

1.3 Epidemiology of diabetes mellitus

Over the past few decades, DM incidence and prevalence have drastically increased globally due to socioeconomic, demographic, environmental, and genetic variables.

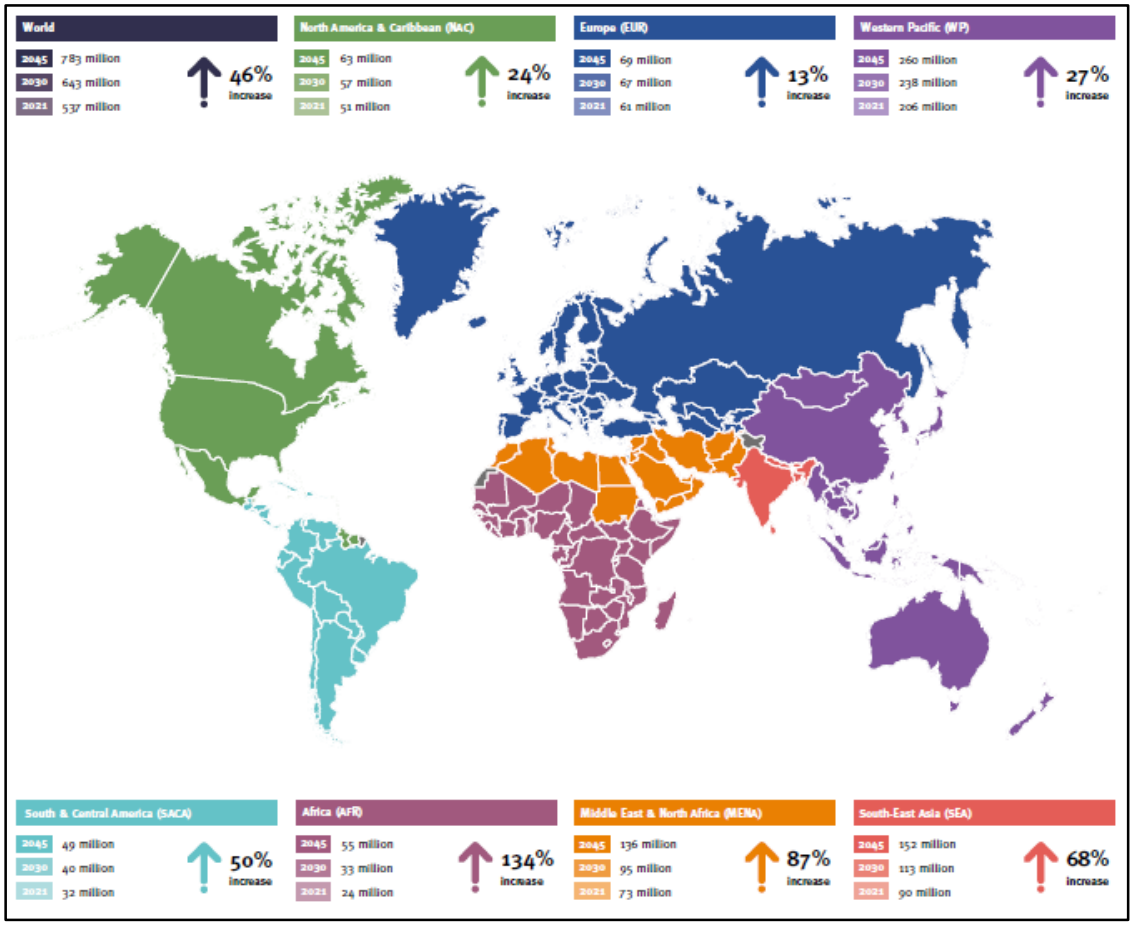

Figure 1.1. Worldwide prevalence of diabetes mellitus (IDF Diabetes Atlas, 2021).

Currently, 536.6 million adults (10.5% of the population) are diagnosed with DM. The total number is predicted to rise to 642.7 million (11.3%) by 2030 and to 783.2 million (12.2%) by 2045 (IDF Diabetes Atlas, 2021). According to the IDF estimates, the worldwide prevalence of DM is summarized in Fig. 1.1. India ranks second in the world, with 74.2 million people suffering from DM, and it is estimated that by 2045, the numbers will reach 124.9 million, respectively, with 43.9 million individuals having undiagnosed diabetes, accounting for 57% of the population (IDF Diabetes Atlas, 2021). While the global prevalence of diabetes in urban areas is 10.8%, it is 7.2% in rural regions (IDF Diabetes Atlas, 2021).

1.4 Diagnostic criteria for diabetes mellitus

Diabetes can be diagnosed based on a measure of fasting blood glucose (eight fasting hours) and on long-term levels of blood glucose (HbA1C proportion). The glycated hemoglobin (HbA1c) test assesses the blood glucose levels over the past three months. However, a more precise method recommended by the WHO and IDF for measuring the level of glycemia is a 2-hour oral glucose tolerance test (OGTT), which also allows for the detection of IFG and IGT.

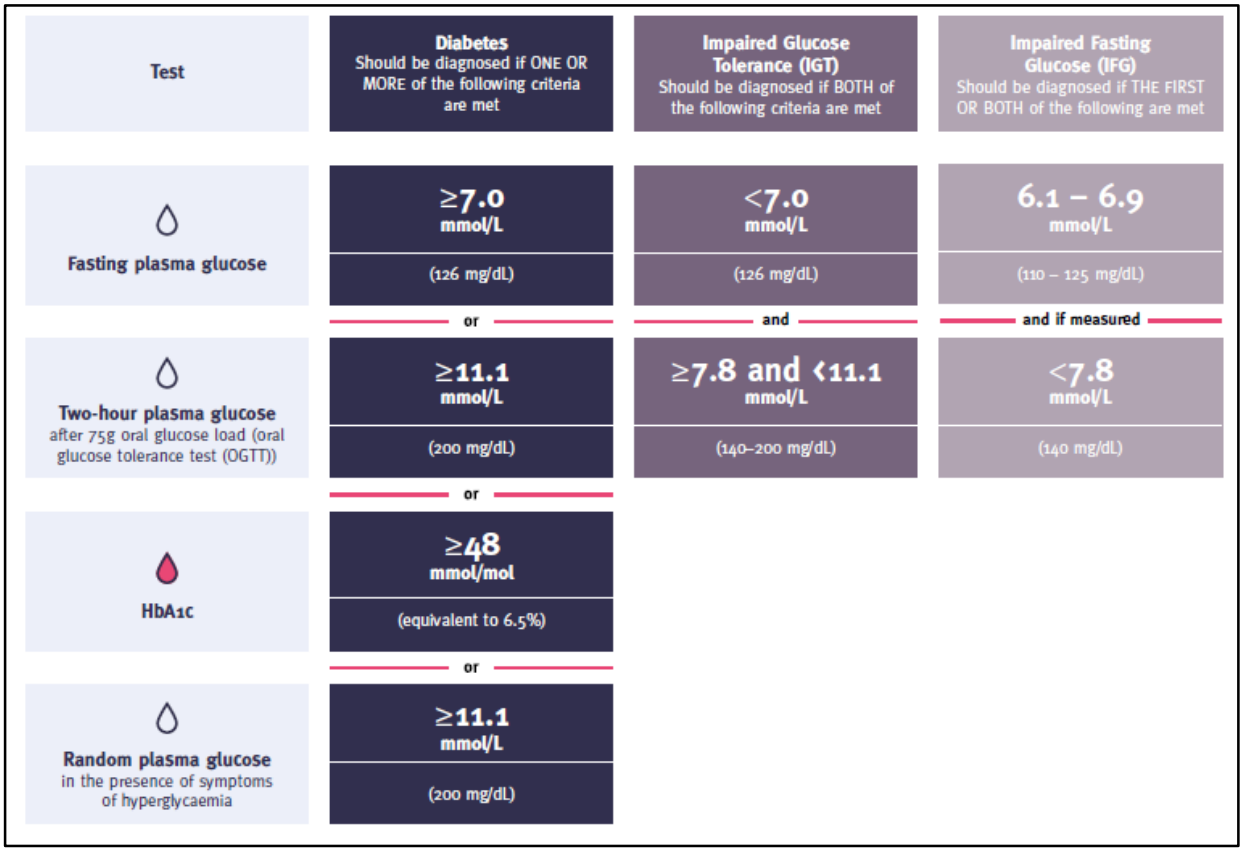

Figure 1.2. Diagnostic criteria for diabetes mellitus (IDF Diabetes Atlas, 2021).

These (IFG and IGT) are two distinct starting states that predispose an individual to diabetes; as a result, they are also known as individuals with pre-diabetes, where the blood glucose concentration is higher than normal fasting blood glucose levels but lower than the diagnosis of diabetes. IGT, IFG, and diabetes diagnostic criteria are summarized in Fig. 1.2. In the presence of symptoms such as excessive urination, thirst, and unexplained weight loss, a random venous blood glucose level of 11.1 mmol/l or a fasting blood glucose level of 7.0 mmol/l (whole blood 6.1 mmol/l or HbA1c 6.5%) allows for a diagnosis of T1D without an OGTT.

1.5 Pathogenesis of diabetes mellitus

Loss of functional β-cell mass is a critical factor in the development of both T1D and T2D (Cnop et al., 2005). T1D and T2D have fundamentally different pathogeneses, which have diverse effects on early β-cell dysfunction (immune-mediated in T1D and metabolic dysregulation-mediated in T2D) and β-cell loss (immense versus mild- to modest) (Eizirik et al., 2020). Cytokines like interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) can promote β-cell dysfunction and death in T1D (Ramos-Rodriguez et al., 2019). In contrast, the pathogenesis of T2D is more complex, as hyperglycemia, hyperinsulinemia, and free fatty acids (FFAs) may induce oxidative stress, Endoplasmic reticulum (ER) stress, and inflammation, which in turn impair the β-cell function and survival. Therefore, comprehending the underlying mechanisms responsible for β-cell failure is pertinent for preventing or reversing DM.

1.5.1 Pathogenesis of type 1 diabetes (T1D)

T1D is an autoimmune disease caused by autoreactive T lymphocytes ($CD8^+$ T cells) that recognize and target the specific antigens expressed on the β-cell surface in the context of HLA class I mediated β-cell apoptosis and dysfunction, and result in hyperglycemia and hyperglycemia-related complications and a lifelong necessity for exogenous insulin therapy (Benninger et al., 2018). T-lymphocyte mediated insulitis, followed by the presence of one or more types of autoantibody (AAb) against insulin, glutamic acid decarboxylase (GAD), protein tyrosine phosphatase IA-2 or IA-2β, and zinc transporter 8 (ZnT8) indicate the immunological onset of T1D (Diabetes Care, 2022; Dayan et al., 2019; Beik et al., 2020). For T1D, a three-stage classification system has been proposed. Stage 1 is defined by the presence of more than one type of AAb (i.e., β-cell autoimmunity) in normoglycemic individuals. Stage 2 is distinct by dysglycemia in the presence of β-cell autoimmunity. Both the stages are asymptomatic.

Stage 3, however, is marked by the clinical presentation with symptoms of hyperglycemia (polyuria, polydipsia, enuresis, weight loss, & blurred vision) and, in some instances, diabetic ketoacidosis (DKA) or diabetic hyperosmolar syndrome (Diabetes Care, 2022; Dayan et al., 2019). T1D results from a complex interaction between invading or resident macrophages and T-cells ($CD8^+$ and $CD4^+$). This interaction leads to the release of chemokines and cytokines (IL-1β and IFN-γ) in the islet microenvironment. Furthermore, pro-inflammatory cytokines disrupt the β-cells' metabolic and electrical activity, insulin granule synthesis and content, gap junction coupling, excessive generation of reactive oxygen species (ROS), and activation of caspases (Benninger et al., 2018; Vilas-Boas et al., 2021). These pro-apoptotic signals, degradation products of insulin, oxidative stress, injury, or dying β-cells, further attract and activate immune cells towards the islets (Gonzalez-Duque et al., 2018; Thomaidou et al., 2018 Eizirik et al., 2009). T1D is a highly heterogeneous disease as this interaction depends on the host's genetic predisposition, age, and environmental factors, such as viral infections and diet (Ilonen et al., 2019; DiMeglio et al., 2018; Op de Beeck et al., 2016). The interaction between immune cells and β-cells can lead to inflammation in the local area, also known as insulitis, and ultimately cause β-cell dysfunction and death mainly through apoptosis (Eizirik et al., 2009; DiMeglio et al., 2018; Todd, 2010). Additionally, the normal physiological processes that reduce the immune response and restore function may be disrupted (Colli et al., 2018; Martinov and Fife, 2020).

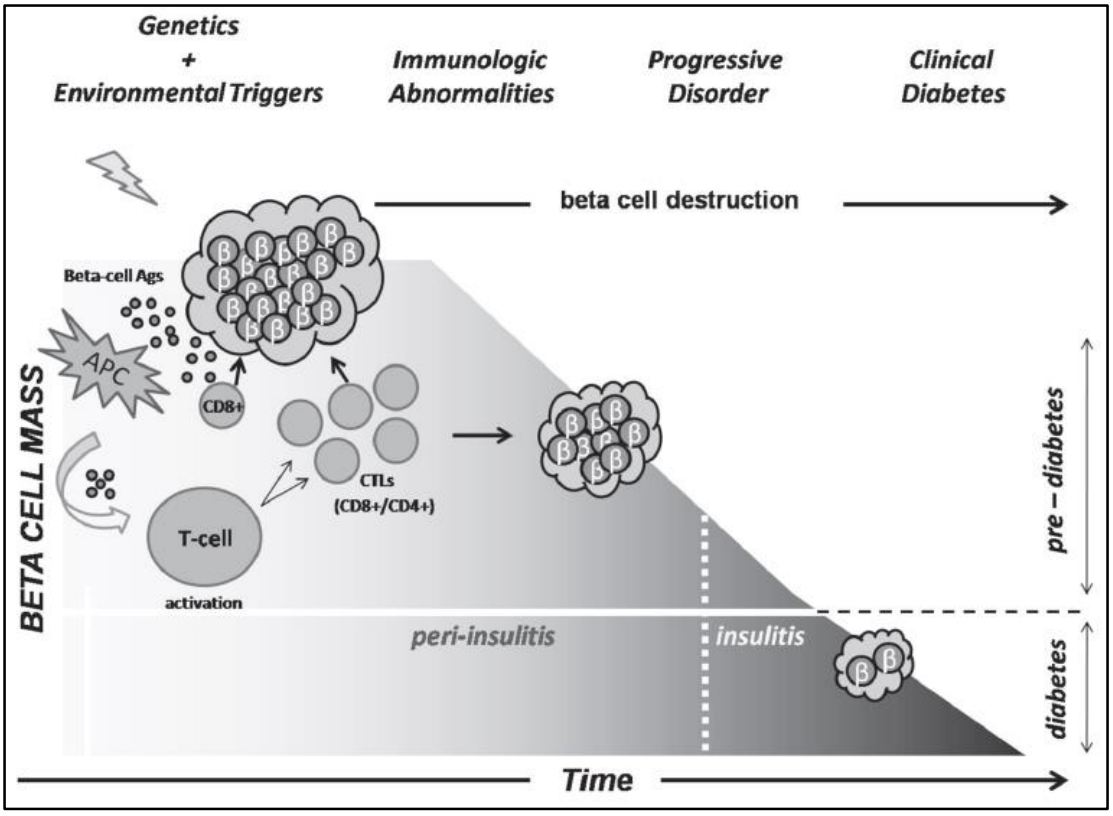

Figure 1.3. Proposed mechanism for the pathogenesis of T1D. Both genetic and environmental factors are essential for releasing β-cell antigens (Ags). β-cell antigens (Ags) are presented by antigen presenting cells (APCs) directly or *via* the cytokine activation cascade to β-cell reactive T-cells. Upon activation of T-cells ($CD4^+$ and/or $CD8^+$), an inflammatory response against pancreatic β-cells (insulitis) develops. The onset of diabetes coexists with a progression from peri-insulitis to severe insulitis. Increased β-cell destruction and decreased β-cell mass result in the transition from pre-diabetes to overt clinical diabetes (Dirice et al., 2011).

According to Sims and DiMeglio (2019), certain T1D patients display evidence of β-cell dysfunction, like decreased C-peptide release in response to glucose and increased levels of proinsulin-insulin ratios in circulation. These findings suggest that β-cell dysfunction might occur before the autoimmune attack in T1D or result from a previous resolved autoimmune episode. T1D incidence in children is doubling every 25 years (Patterson et al., 2019) and is associated with a decrease in life expectancy of around 11-12 years (Huo et al., 2016). Currently, no therapeutic approaches are available to prevent or cure T1D (DiMeglio et al., 2018; Greenbaum et al., 2018), a recent study in stage 2 patients, using a monoclonal antibody against CD3 to target CD8+ T cells, delayed, but did not prevent the disease onset by approximately two years (Herold et al., 2019).

1.5.1.1 Role of genetic factors in T1D

T1D is strongly linked to HLA DR and DQ. HLA DR and DQ are cell surface receptors that present antigens to T-lymphocytes. Both DR and DQ are alpha-beta heterodimers. The *DRA* locus encodes the DR alpha chain, while the *DRB* locus encodes the DR beta chain. Similarly, the *DQA1* and *DQB1* loci encode the DQ molecule's alpha and beta chains, respectively (Redondo et al., 2018). The *DR* and *DQ* loci are strongly linked to one another and, to a lesser extent, to other *HLA* loci. In addition to the *DRB1* and *DQ* alleles, other genetic variables may increase the risk of T1D. For instance, *DRB3*, *DRB4*, and *DRB5* alleles alter the risk conferred by *DRB1* (Zhao et al., 2016). T1D has been linked to *HLA-DR3-DQ2* and *HLA-DR4-DQ8* haplotypes, either alone or in combination. The association between HLA haplotypes and T1D appears to reflect a link between *HLA* haplotypes and the formation of the first islet autoantibody. For instance, Glutamic acid decarboxylase autoantibodies (GADAb) were often the first autoantibody in children homozygous for the *HLA-DR3-DQ2* haplotype, whereas insulin autoantibodies were typically the first autoantibody in children with the *HLA-DR4-DQ8* haplotype. Nevertheless, Class II genes do not fully explain the link between HLA and T1D; HLA Class I genes (A, B, and C) also influence T1D risk (Erlich et al., 2013; Noble et al.,

2002), as well as the age of onset, in interaction with Class II genes (*DR* and *DQ*). The combination of those two haplotypes into the *DR4-DQ8*/*DR3-DQ2* genotype confers the highest risk of T1D, with an average OR of 16 (Ilonen et al., 2016). The idea that other HLA class II molecules have a role in the first trigger of β-cell autoimmunity cannot be ruled out because next-generation sequencing has shown that *HLA-DRB3, HLA-DRB4,* and *HLA-DRB5* are associated with β-cell autoantibodies and an increased risk of T1D (Erlich et al., 2013; Zhao et al., 2016). The insulin gene (*INS*) shows the strongest association with T1D after the HLA locus. Additionally, polymorphisms in the *INS* gene contribute to the genetic aetiology of diabetes, particularly in children with *HLA-DR4-DQ8*, as *INS* rs689 risk genotype A/A and the A allele were more frequent among children with insulin autoantibodies. Protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), another gene with well-studied polymorphisms, also correlates to the risk for insulin autoantibodies (Ilonen et al., 2013).

Currently, 58 genomic regions show significant genome-wide evidence for T1D association and about 50 genes are proposed to be potential causal disease genes. Results from a genome-wide association study (GWAS) meta-analysis and SNP profiling studies indicate that a little over 100 SNPs can mark statistically significant associations, accounting for around 80% of its heritability (Barrett et al., 2009; Groop et al., 2014). Further following SNPs are strongly associated with HLA and non-HLA regions and have recently been tested for prediction of T1D: rs2476601 in *PTPN22*; rs1004446 in *INS*; rs3087243 in cytotoxic T-lymphocyte associated protein (*CTLA4*); rs11203203 and rs9976767 in ubiquitin-associated and SH3 domain-containing protein A (*UBASH3A*); rs2292239 in erb-b2 receptor tyrosine kinase 3 (*ERBB3*); rs3184504 in SH2B adapter protein 3 (*SH2B3*); interleukin-2 receptor subunit alpha (*IL2Rα*), interferon-induced helicase (*IFIH1*), basic leucine zipper transcription factor 2 (*BACH2*); interleukin-27 (*IL27*); BCL2 Associated Agonist Of Cell Death (*BAD*); C-Type Lectin Domain Containing 16A (*CLEC16A*), regulator of G-protein signaling 1 (*RGS1*), and Cathepsin H (*CTSH*) (Pociot and Lernmark et al., 2016; Redondo et al., 2018). Several pathogenic mechanisms may be involved, in addition to genetic variables that directly influence immune response and β-cell function. For example, it is becoming evident that having a high BMI contributes to the development of T1D (Ferrara et al., 2017), and the genetics of obesity is emerging as an additional factor. Similar to T2D, certain T1D patients may be affected by genes that control T2D's pathogenic mechanisms (Redondo et al., 2014; Redondo et al., 2017). Thus, the crucial role of genetics is to enhance prediction so that strategies to avoid such disease in at-risk persons can be created and implemented.

1.5.2 Pathogenesis of type 2 diabetes (T2D)

The pathogenesis of T2D is more complex, and it involves different degrees of β-cell failure and insulin resistance. Obesity-induced insulin resistance and insulin secretion defects are the major risk factors for T2D (Klöppel et al., 1985; Kahn, 2003). The failure of insulin-stimulated glucose uptake in muscles is a crucial deformity in T2D. In addition, successive resistance to insulin's antilipolytic activities promotes increased lipolysis and fatty acid release in certain fat depots. These fatty acids diminish the insulin's ability to suppress glucose production in the liver while promoting a continuous increase in insulin-stimulated fatty acid synthesis. Therefore, dysregulation of carbohydrate and lipid metabolism encourages the progression of insulin resistance (Lee et al., 2022). Pancreatic β-cells compensate for the insulin-resistance by increasing basal and postprandial insulin secretion, further exacerbating insulin resistance. At some point, the β-cells can no longer compensate for hyperglycemia and insulin resistance, leading to impaired glucose homeostasis and the development of glucose intolerance. Each year, around 5%-10% of glucose-intolerant patients develop pre-diabetes, which worsens as insulin resistance grows. Obesity, genetic predisposition, high caloric diets, old age, and sedentary lifestyle are the key risk factors for T2D (Zheng et al., 2018). These risk factors can trigger both β-cell failure and insulin resistance, leading to a four-fold increase in cases over the last four decades (NCD Risk Factor Collaboration, 2016).). The aforementioned risk factors can worsen both β-cell failure and insulin resistance, leading to the later stages of the disease, where high doses of exogenous insulin may be required. For T2D management, many drug classes are available, none of which have been shown to modify the progressive decline in β-cell function over time. Cytokine-induced inflammation, obesity, insulin resistance, high saturated fat intake, and excessive free fatty acid consumption are the main factors that insult the β-cells. In addition to the decline in β-cell function and mass, the integrity of the islets is also affected, which could diminish its signaling. The factors implicated in the pathogenesis of T2D are summarized in Fig. 1.4.

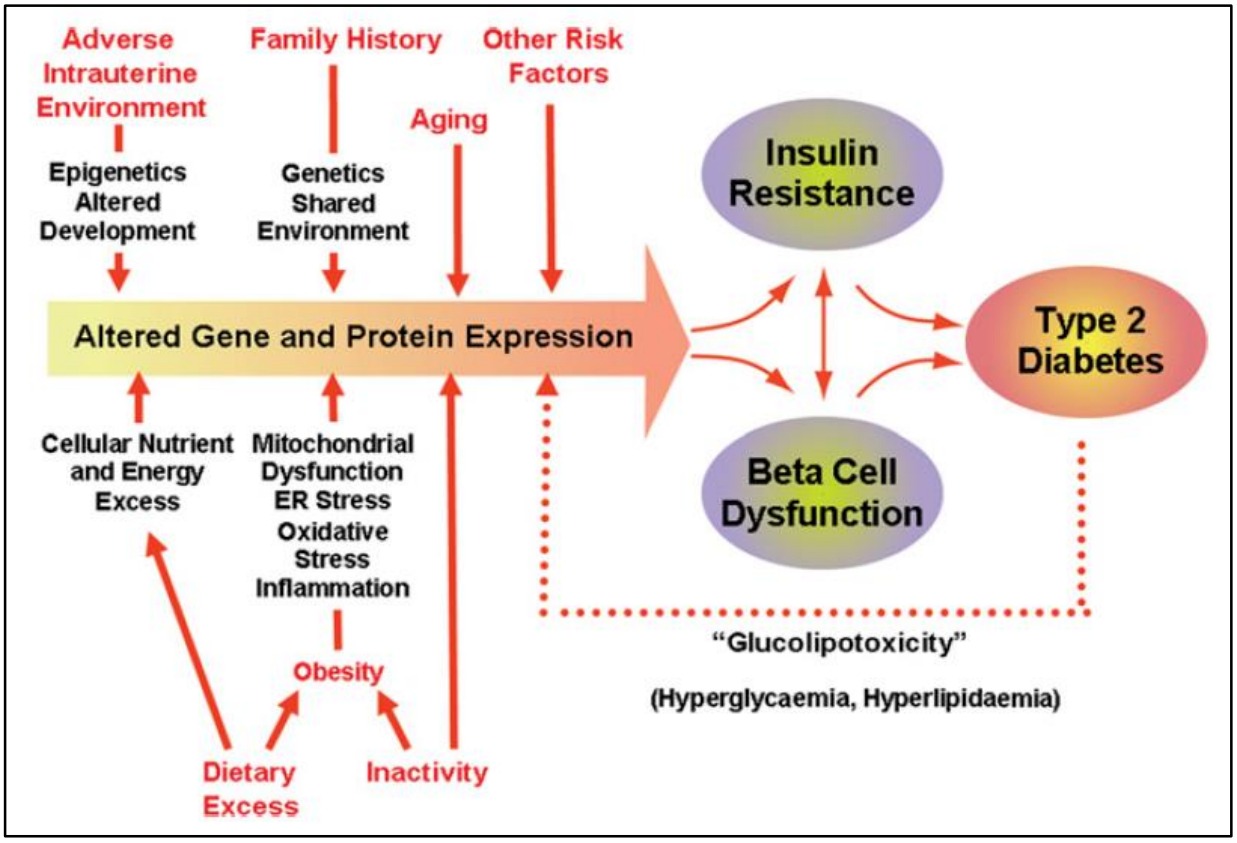

Figure 1.4. Role of genes and environmental factors in developing obesity-insulin resistance and T2D. The interaction of genes that affect body adiposity with environmental factors can lead to the progression of obesity-induced insulin resistance and β-cell dysfunction, resulting in chronic hyperglycemia and hyperlipidemia. The pancreatic islet response to this new environment is likely to differ among individuals with varying genetic susceptibility, including inflammatory stress, ER stress, metabolic and oxidative stress (e.g., glucotoxicity, lipotoxicity, and glucolipotoxicity), amyloid stress, and islet cell integrity loss. These interconnected stressors could augment, by course, and promote β-cell dysfunction, if left untreated. 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

A complex interplay of genetic and environmental/lifestyle factors determine an individual's likelihood of developing T2D. These genes vary in strength and location of the interaction, and they differ in the general population according to race and ethnicity. Barnet (1981) concluded the higher concordance rate in identical twins than in non-identical twins regardless of the age of the onset of the disease. Similarly, Medici et al. (1999) have demonstrated that monozygotic twins showed a 76 % concordance for T2D with a 96 % concordance for impaired glucose tolerance, suggesting an essential role of genotype. Furthermore, having a family history of T2D doubles an individual's chance of acquiring the disease (Medici et al., 1999). The risk of developing the disease is determined by combining several genetic variants at multiple gene

loci, each of which confers a marginal increase in the disease risk, as T2D is a polygenic disease (Lyssenko and Laakso, 2013). Therefore, T2D differs from monogenic forms of diabetes (maturity-onset diabetes of the young (MODY) and neonatal diabetes (Gloyn et al., 2004). GWAS are the most effective way to identify genes contributing to polygenic disorders based on the association of common genetic variants, i.e., single-nucleotide polymorphisms (SNPs) with a specific trait, such as hyperglycemia, BMI, HbA1c and lipid profile. Further, to generate significant statistical power, GWAS studies involve large cohort sizes (in some studies, >100,000 persons). GWAS has found roughly 75 loci that are associated with T2D susceptibility and related metabolic traits. Of these, 45 loci were found in European, and 29 were found in Asian populations, particularly in East and South Asians (Sun et al., 2014) and some in African population (Hertel et al., 2013; Sanghera et al., 2012; Prasad et al., 2015) (Table 1.1). These genes confer the risk of diabetes by interfering with the following three mechanisms: i) reduction in insulin secretion, ii) impairment in incretin release, and iii) impaired proinsulin-to-insulin conversion. GWAS aims to find SNPs (located in linkage disequilibrium with other variations) those serve as a proxy for disease-associated regions or loci rather than the actual causative variants (Slatkin, 2008). Additionally, the association is discovered in non-coding regions, which control genes to influence disease risk. The SNPs in non-coding regions are designated after the protein-coding genes that are closest to them, although this closeness does not imply causality (Maurano et al., 2012). Thus, finding the gene and a causal variant affecting disease susceptibility is essential. *Ex-vivo* studies have demonstrated the direct influence of a subset of diabetes risk loci on impaired insulin secretion, thereby providing the mechanistic insights into the role of these genetic variants (Rosengren et al., 2012). By prioritizing putative causative genes based on functional data, high-throughput screens also allow the transition from T2D GWAS association findings to individual functional follow-up research (Grotz et al., 2017).

Recent population-based studies suggest a 12-16% rising prevalence of diabetes in India's urban areas. Anjana et al. (2017) have demonstrated that plasma insulin levels are higher in Asian Indians than Europeans, and Asian Indians are more insulin resistant (Anjana et al., 2017). Intriguingly, Indians had higher body fat at any BMI; even when body fat was equal, Indians had more significant insulin resistance than other ethnic groups. These studies suggest that Indians are genetically more prone to diabetes and insulin resistance (Anjana et al., 2017). Furthermore, ethnicity has a significant impact on the distribution of gene polymorphisms, especially cytokine gene polymorphisms (Hoffman et al., 2002). Thus, association studies must

be carried out across various ethnicities to support the relationship of causative genetic variations with their functional link to the disease susceptibility.

Table 1.1. Summary of genetic loci associated with risk of T2D.

Sr. No.	SNP	Gene	Chr.	Ethnicity	Trait/ Association
1.	rs340874	*PROX1*	1	European	Elevated FBG
2.	rs7542900	*SLC44A3*	1p21.3	African	T2D
3.	rs17045328	*CR2*	1q32.2	Asian	T2D
4.	rs12027542	*PCNXL2*	1q42.2	Asian	T2D
5.	rs10923931	*NOTCH2*	1p12	European	Pancreatic organogenesis and regulates cell differentiation.
6.	rs243021	*BCL11A*	2p16.1	European	Affects insulin response towards glucose
7.	rs7593730	*RBMS1*	2q24.2	European	T2D
8.	rs7560163	*RBM43*	2q23.3	African	T2D
9.	rs780094	*GCKR*	2	European	Associated with FBG, fasting insulin and HOMA-IR
10.	rs3923113	*GRB14*	2q24.3	South Asians	Reduced insulin sensitivity.
11.	rs2943641	*IRS1*	2q36.3	European, French	Impaired metabolic profile (Adiposity, IR, dyslipidemia, CVD, adiponectin levels).
12.	rs7578597	*THADA*	2p21	European	Associated with β-cell dysfunction & and reduced β-cell mass and lower β- cell response towards GLP-1.
13.	rs16861329	*ST6GAL1*	3q27.3	South Asians	T2D
14.	rs7630877	*PEX5L*	3q26.33	Asians	T2D
15.	rs3773506	*PLS1*	3q23	Asians	T2D
16.	rs831571	*PSMD6*	3p14.1	Asians	T2D
17.	rs2063640	*ZPLD1*	3q12.3	Asians	T2D
18.	rs11708067	*ADCY5*	3	European	Associated with elevated FBG
19.	rs4607103	*ADAMTS9*	3p14.1	European	Affects insulin response towards glucose
20.	rs4402960	*IGF2BP2*	3q27.2	European	T2D
21.	rs358806	*NR*	3p14	European	T2D
22.	rs1801282	*PPARG*	3p25.2	European	Effect on IR
23.	rs1801214	*WFS1*	4p16.1	European	Effect Insulin response
24.	rs6815464	*MAEA*	4p16.3	Asians	T2D
25.	rs4457053	*ZBED3*	5q13.3	European	T2D
26.	rs1048886	*C6orf57*	6q13	Asians	T2D
27.	rs1535500	*KCNK16*	6p21.2	Asians	T2D
28.	rs9470794	*ZFAND3*	6p21.2	Asians	T2D

29.	rs7754840	*CDKAL1*	6p22.3	European, Icelandic	Effect Insulin response
30.	rs896854	*TP53INP1*	6q22.1	European	T2D
31.	rs9472138	*VEGFA*	6p21.1	European	T2D
32.	rs7636	*ACHE*	7q22.1	Asians	T2D
33.	rs972283	*KLF14*	7q32.3	European	Associated with BMI, insulin, glucose and cholesterol.
34.	rs972283	*DGKB*	7	European	Associated with elevated FBG
35.	rs4607517	*GCK*	7	European	Associated with elevated FBG and HbA1c.
36.	rs864745	*JAZF1*	7p15.1	European	Effect Insulin response
37.	rs13266634	*SLC30A8*	8q24.11	European, French	Associated with fasting proinsulin levels.
38.	rs7041847	*GLIS3*	9p24.2	Asians	T2D
39.	rs17584499	*PTPRD*	9p24.1	Asians	T2D
40.	rs13292136	*TLE4*	9	European	T2D
41.	rs17584499	*PTPRD*	9	Taiwanese	Associated with increased HOMA-IR
42.	rs10811661	*CDKN2A/B*	9	European	Associated with CVD Effect Insulin response
43.	rs13292136	*CHCHD9*	9q21.31	European	T2D
44.	rs1802295	*VPS26A*	10q22.1	South Asians	T2D
45.	rs6583826	*KIF11*	10q23.33	Asians	T2D
46.	rs10741243	*TCERG1L*	10q26.3	Asians	T2D
47.	rs12779790	*CDC123*	10p13	European	Affects insulin response towards glucose
48.	rs1111875	*HHEX*	10q23.33	European, French	Affects insulin release and insulin sensitivity.
49.	rs7903146	*TCF7L2*	10q25.2	European	Affects insulin and glucagon secretion
50.	rs7107217	*TMEM45B*	11q24.3	African	T2D
51.	rs2722769	*GALNTL4*	11p15.3	African	T2D
52.	rs1552224	*ARAP1*	11q13.4	European	Associated with lower proinsulin levels and β-cell function
53.	rs10830963	*MTNR1B*	11q14.3	European	Associated with increased FBG and reduced β-cell function.
55.	rs2237892 rs2237897	*KCNQ1*	11p15.5 11p15.4	Japanese, Asian, European	Affects Insulin responsiveness
56.	rs5219	*KCNJ11*	11p15.1	European	Impairs insulin secretion
57.	rs1531343	*HMGA2*	12q14.3	European	Affects insulin action
58.	rs1153188	*DCD*	12q13.2	European	T2D
59.	rs7957197	*HNF1A*	12q24.31	European	T2D
60.	rs7961581	*TSPAN8*	12q21.1	European	Affects insulin responsiveness

61.	rs1359730 rs1359790	*SPRY2*	13q31.1	Asians Japanese	Associated with body fat percentage
62.	rs730570	*C14orf70*	14q32.2	Mexican	T2D
63.	rs2028299	*AP3S2*	15q26.1	South Asians	T2D
64.	rs7178572	*HMG20A*	15q24.3	South Asians	T2D
65.	rs11071657	*C2CD4A/B*	15q22.2	Japanese	Associated with FBG and proinsulin levels.
66.	rs1436955	*C2CD4B*	15q22.2	Asians	T2D
67.	rs11634397	*ZFAND6*	15q25.1	European	T2D
68.	rs8042680	*PRC1*	15q26.1	European	T2D
69.	rs8050136, rs9939609	*FTO*	16q12.2	British, European	Affects BMI
70.	rs16955379	*CMIP*	16q23.2	Asians	T2D
71.	rs17797882	*WWOX*	16q23.2	Asians	T2D
72.	rs391300	*SRR*	17p13.3	Taiwanese, Asians	Affects insulin and glucagon secretion.
73.	rs757210	*HNF1B*	17q12	European	T2D
74.	rs10460009	*LPIN2*	18p11.31	Asians	T2D
75.	rs472265	*PAPL*	19q13.2	Asians	T2D
76.	rs3786897	*PEPD*	19q13.11	Asians	T2D
77.	rs2833610	*HUNK*	21q22.11	Asians	T2D
78.	rs4812829	*HNF4A*	20q13.12	South Asians	Impairs insulin secretion
79.	rs5945326	*DUSP9*	Xq26	European	Insulin resistance

[Abbreviations: Chr: chromosome; CVD: cardiovascular disease; FG: fasting glucose; GI: gastrointestinal; IR: insulin resistance; IS: insulin secretion; T2D: type 2 diabetes]

1.5.2.2 Role of cytokines, oxidative stress, ER stress and inflammation in T2D

The role of oxidative stress and inflammation is well-known in DM pathogenesis. The inflammatory condition triggers the development of obesity-induced insulin resistance (IR) and DM through a very complex mechanism consisting of several kinases and interacting signaling pathways and generating ROS such as hydrogen peroxide and superoxide anions (Mahmoud and Al-Ozairi, 2013; Patel et al., 2013). Pro-inflammatory cytokines cause β-cell death by inducing mitochondrial stress and other responses (Gurgul-Convey et al., 2011; Cnop et al., 2005). Cytokines secreted by immune cells infiltrating the pancreas are crucial mediators of β-cell destruction (Lin et al., 2012). As a result of an excess of glucose and oxidative phosphorylation, mitochondria generate more reactive oxygen species (ROS) and experience more oxidative stress. Endoplasmic reticulum (ER) stress is critical in oxidative stress, since it is also a source of ROS. Because of the tight interconnection between mitochondria and ER via mitochondrial-associated membranes (MAMs), ROS produced in mitochondria also induces ER stress. As a result of this vicious cycle, cellular stress and mitochondrial malfunction occurs

(Burgos-Morón et al., 2019). As mitochondria are involved in insulin release (Ježek et al., 2022) and pancreatic β-cells are exposed to hyperglycemia, they are more vulnerable to oxidative stress and mitochondrial dysfunction. Failure of the ER's adaptive capacity and increased unfolded protein response activation may prompt autophagy as self-defense and inflammatory processes are linked to β-cell death in T2D. Additionally, β-cells' limited glycolytic capacity can allow more ROS generation, and the emerging oxidative stress can decouple glucose sensing from insulin secretion (Robertson, 2004). For, endogenous incretins that potentiate "glucose-stimulated insulin secretion (GSIS)", β-cells are highly dependent on ATP synthesis, and they are sensitive to excess ROS due to their inherently low antioxidant enzyme expression (Simmons, 2007). Nutrient imbalance or reduced availability to β-cells, increased ROS generation, decreased ATP synthesis, and insufficient antioxidant balance may all predispose to β-cell death/dysfunction (Reusens et al., 2011). Furthermore, glucotoxicity and lipotoxicity have been linked to the onset of cellular stress in β-cells and peripheral tissues. Moreover, factors responsible for insulin resistance and β-cell loss are summarized in Fig 1.5.

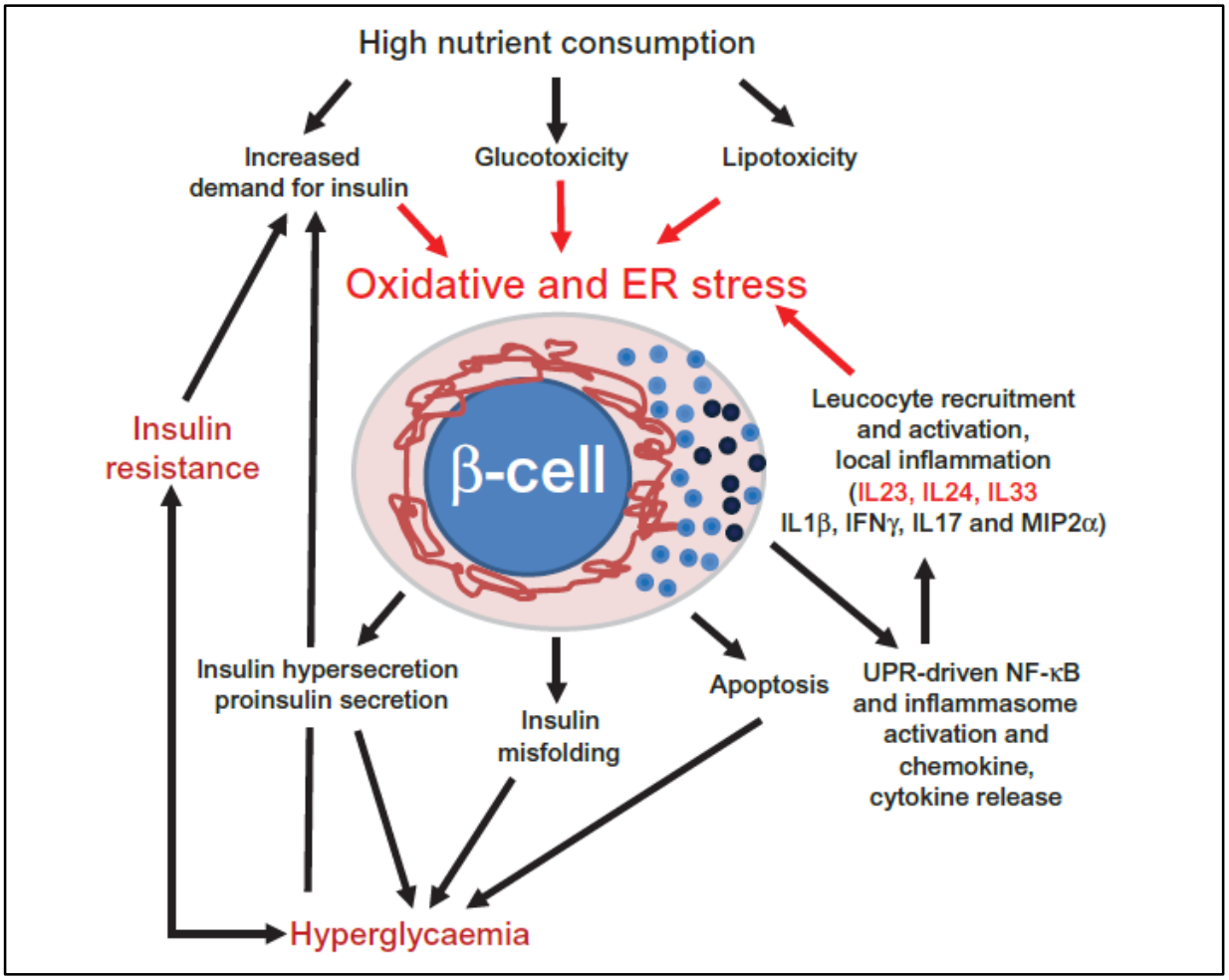

Figure 1.5. Cellular stress, insulin resistance and β-cell apoptosis. Glucotoxicity and lipotoxicity lead to ER stress, oxidative stress, and inflammation by generating ROS in the ER

and mitochondria. Oxidative stress and ER stress promote chemokine production, which activates inflammatory cells in the pancreatic islet microenvironment, leading to disturbed β-cell secretory pathway function. Insulin resistance in the peripheral tissues keeps increasing due to the persistent glucotoxicity and lipotoxicity, thus requiring more insulin synthesis and secretion. Acute ER stress activates the unfolded protein response (UPR), which leads to suppression of the insulin production and secretion. As diabetes progresses, insulin production diminishes, and β-cell apoptosis rises, thereby exacerbating the glycemic control (Hasnain et al., 2016).

1.5.2.3 Obesity-induced inflammation and insulin resistance

Chronic inflammation characterized by macrophage infiltration has been shown to occur predominantly in the adipose tissue or liver of an obese individual, in which activation of immune cells is closely associated with insulin sensitivity and can initiate a state of insulin resistance (IR) by modulating adipose tissue's glucose and lipid metabolism (Tateya et al., 2013). Macrophages can be classified as classically activated M1 (pro-inflammatory) and alternatively activated M2 (anti-inflammatory). In the context of insulin action, M2 macrophages sustain insulin sensitivity by secreting IL-4 and IL-10, while M1 macrophages promote insulin resistance by secreting pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IFN-γ, leptin) and enhancing the inflammatory response. Thus, the activation status of infiltrating macrophages plays an essential role in the progression of metabolic disorders (Tateya et al., 2013; McArdle et al., 2013). Various dynamic interactions of immune cells control the polarization of M1/M2. In a lean state, TH2 cells, Treg cells, natural killer T cells, or eosinophils contribute to the activation of M2 phenotype by secreting IL-4 or IL-10. In contrast, obesity favors altering the constituent immune cells, in which TH1 cells, B cells, neutrophils, or mast cells induce M1 phenotype activation by increasing the secretion of TNF-α and IFN-γ. Concomitantly, excess energy intake leads to adipocyte hypertrophy, and hyperplasia promotes increased secretion of TNF-α, and free fatty acids further contribute to the activation of the M1 phenotype (Fig 1.6). Adipose tissue (AT) dysfunction also impaired hepatic and skeletal muscle glucose homeostasis, leading to systemic IR and, eventually, the development of T2D. Therefore, maintaining the M2 phenotype over the M1 phenotype is desirable and crucial for proper glucose and lipid synthesis and subsequent secretion (Tateya et al., 2013).

Odegaard et al. (2008) have demonstrated that HFD intake induces the polarization of Kupffer (liver resident macrophage) cells towards the M1 phenotype, implicated in the development of obesity-induced insulin resistance and fatty liver disease and increased activation of c-Jun N-

terminal protein kinase (JNK1) (Odegaard et al., 2008). TNF-α produced by liver M1 macrophages can increase glucose output through gluconeogenesis and decrease glycogen content. By inhibiting intracellular lipases and making "fatty acids available for triacylglycerol (TAG)" synthesis, it can also contribute to increased lipid biosynthesis and storage (Gao et al., 2010). Under chronic hyperglycemia/hyperlipidemia conditions, along with proinsulin, (pro) islet amyloid polypeptide (IAPP) synthesis increases in β-cells and reaches threshold levels that allows the pro-apoptotic IAPP oligomers' formation (Montane et al., 2012). This induces IL-1β secretion to recruit macrophages and augment local islet inflammation (Masters et al., 2010). These inflammatory reactions lead to hyperglycemia and dyslipidemia, a hallmark of obesity-induced insulin resistance and T2D.

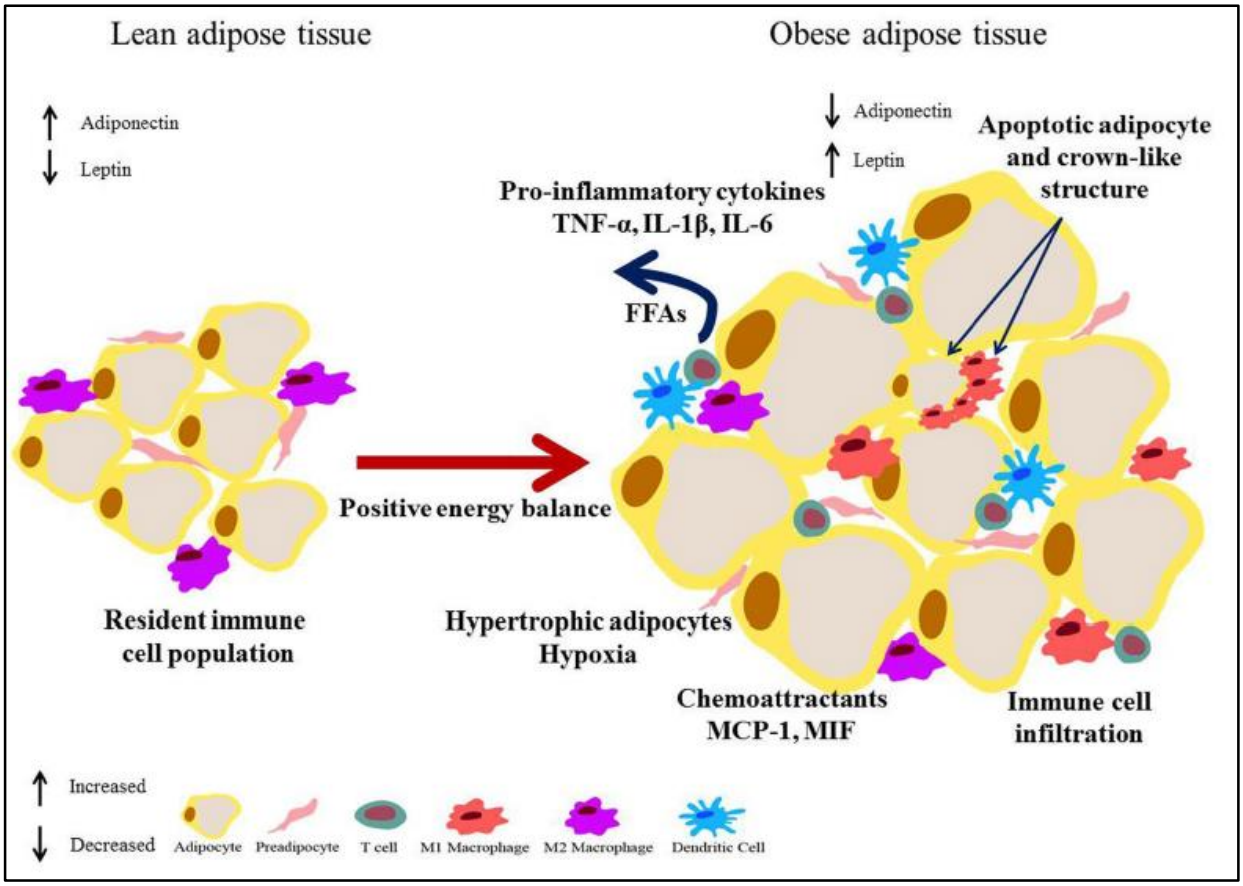

Figure 1.6. Obesity-induced macrophage infiltration into adipose tissue convicts inflammation and insulin resistance. Excess energy intake and lack of exercise-induced adipocyte hypertrophy and hyperplasia induce the secretion of chemoattractants, such as Monocyte chemoattractant protein-1 (MCP-1), which recruits circulating immune cells to adipose tissues. Adipocytes, pre-adipocytes, and infiltrating immune cells secrete pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6, which polarize macrophages towards the M1 phenotype and drive an inflammatory T-cell population. All together it contributes to low-grade inflammation in adipose tissue and responsible for the imbalance of pro-inflammatory (leptin, resistin, IL1-β, TNF-α)/anti-inflammatory (vaspin, adiponectin, and omentin) adipokines, which impair the glucose and lipid metabolism. This environment

negatively impacts the insulin signaling pathway and promotes insulin resistance (McArdle et al., 2013).

1.5.2.3.1 Insulin signaling and mechanism of insulin resistance:

Insulin is a pleiotropic hormone, and its secretion is enhanced in response to increased circulating glucose and amino acids. Insulin mediates its anabolic effects via insulin receptor (INSR) across various insulin target tissues through several intracellular signaling cascades. In skeletal muscle, insulin promotes glucose uptake by stimulating the translocation of glucose transporter 4 (GLUT4) to the plasma membrane. In the liver, insulin inhibits the expression of key gluconeogenic enzymes. In the adipose tissue, insulin signaling results in decreased hormone-sensitive lipase activity, and this anti-lipolytic effect inhibits the free fatty acid (FFA) efflux from adipocytes (Yu et al., 2002; Lee et al., 2006; Pickersgill et al., 2007).

The INSR is a heterotetramer composed of two α-subunits and two β-subunits connected by disulphide bonds. Insulin binds to the insulin receptor's α-subunit and activates the tyrosine kinase in the β-subunit. When the insulin receptor tyrosine kinase is activated, it promotes auto-phosphorylation of the β-subunit, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity (White et al., 1988), thereby resulting in the recruitment and phosphorylation of intracellular adapter proteins, insulin receptor substrate (IRS). There are thirteen distinct isoforms of IRS that have been identified and characterized. Among these isoforms, 1 and 2 have been extensively studied because they are present in various cell types and are predominantly activated in skeletal muscle (Corcoran et al., 2007). These two isoforms are responsible for approximately 75% of the insulin-stimulated glucose uptake in the body (Shulman et al., 1990; Corcoran et al., 2007). When IRS1 is phosphorylated, along with IRS2 to a lesser extent, they bind to the regulatory subunit p85 of the “phosphoinositide 3-kinase (PI3K) lipid kinase” and activate it. This results in the conversion of “phosphatidylinositol 3,4-bisphosphate (PIP2) to PI(3,4)P2 and phosphatidylinositol 3,4,5 triphosphate (PIP3)” through the catalytic subunit p110. This conversion then leads to the sequential activation of “3-phosphoinositide-dependent protein kinase 1 (PDK1)”, which in turn recruits and phosphorylates protein kinase B (pAkt) at the plasma membrane. Atypical protein kinase C (aPKC) activated by PDK1, which regulates the glucose metabolism (White, 2003). The downstream effect of this interaction is the activation of pAkt,, which has more than 100 substrates that regulate diverse cellular processes, including glucose homeostasis, cell proliferation, differentiation, endocytosis, and survival (Manning and Cantley, 2007). Among the three isoforms of Akt, Akt2 is the most abundant in insulin-

sensitive tissues. Cho et al. (2001) demonstrated that removing Akt2 in knockout mice resulted in increased insulin resistance, demonstrating the crucial role of Akt2 in regulating glucose homeostasis. Akt plays a critical role in regulating the translocation of glucose transporter type 4 (GLUT4) vesicles to the plasma membrane, which is essential for facilitating the uptake of free glucose in insulin-sensitive tissues (Taniguchi et al., 2006; Henriksen et al., 2011). The second pathway activated by pIRS is the Ras-mitogen-activated protein kinase (MAPK) pathway, which mediates gene expression and interacts with the PI3K-AKT pathway to control cell growth and differentiation (Taniguchi et al., 2006).

Although insulin signaling is well studied, the molecular mechanisms through which insulin resistance develops are still unclear. Changes in insulin receptor expression, ligand binding, phosphorylation, and kinase activity alter downstream insulin signaling, which can result in insulin resistance. Insulin receptor gene (*INSR*) mutations are infrequent, but more than 30 *INSR* mutations have been reported to cause insulin receptor dysfunction, and these mutations may induce insulin resistance with polygenic defects in its downstream signaling (Hegele, 2003). Furthermore, mutations in the *DM1 kinase* gene result in defective alternative splicing of *INSR* (Savkur et al, 2001), while mutations in the high-mobility group A1 (*HMGA1*) gene reduce the *INSR* expression, leading to insulin resistance (Chiefari et al., 2011). In addition, IRS protein mutations and serine hyperphosphorylation are predominantly linked with the development of insulin resistance, as they reduce the IRS interaction with PI3K. Araki et al. (1994) have demonstrated that homozygous disruption of *IRS1* transcription resulted in mild insulin resistance, whereas IRS2-knockout mice displayed severe insulin resistance (Kubota et al., 2000). In rodents and humans, the decreased IRS protein levels contribute to insulin resistance (Shimomura et al., 2000).

Inflammatory adipokines, including TNF-α, IL-6, and IL-1β trigger the suppressor of cytokine signaling 1/3 (SOCS1/3) to increase the degradation of IRS1/2 by activating E3 ubiquitin ligase, which decreases the production of IRS2 in the presence of excess insulin (Rui et al., 2002). The phosphorylation of IRS on serine residues is another mechanism for insulin resistance. IRS contains several serine residues that are phosphorylated by kinases such as cJunN-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), protein kinase Cz (PKCz), and p70S6K. (Boura-Halfon and Zick, 2009). As Ser-307 is located in the IRS's PTB domain, phosphorylation of IRS on Ser-307 is a typical inhibitory signal to suppress insulin signaling (Hirosumi et al., 2002). Furthermore, insulin-activated ERK phosphorylates IRS1 on

Ser-612 to attenuate AKT activation (Bard-Chapeau et al., 2005).

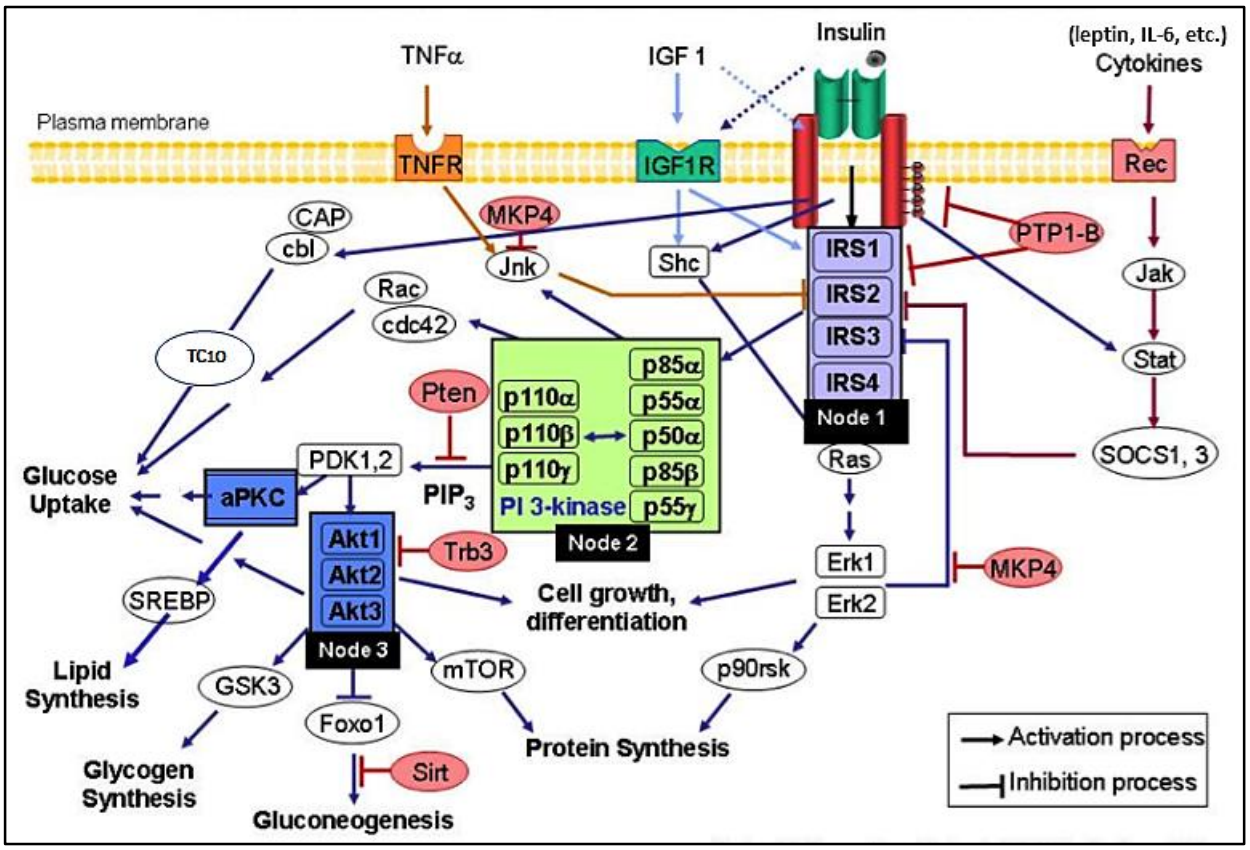

Figure 1.7. Direct interaction of insulin signaling and inflammatory pathways and their role in insulin resistance. The insulin signaling cascade branches into two main pathways PI3K/AKT and Ras/MAPK, which mediate various cellular processes, including cell proliferation, differentiation, endocytosis, metabolism, survival, and glucose homeostasis. Stimulation of the NF-κB and AP-1 Fos/Jun inflammatory pathways results in the activation of the serine kinases, Ikkb and Jnk1, which reduce the signaling ability of IRS1. Additional inflammation-related negative regulators of IRS proteins include the Socs proteins and NO, which are induced in inflammation and promote IRS degradation. High nutrients can modulate IRS1, PI3K, and AKT phosphorylation, leading to the insulin resistance by altering the insulin-signaling cascade (De Meyts, 2016).

Additionally, several amino acid alterations in T2D patients, such as Gly972Arg (Martínez-Gómez et al., 2011) in IRS1 proteins, affect the protein function, but some of these modifications have been debated. Increased insulin resistance is caused by hyperphosphorylation of Serine at Ser302, Ser307, Ser612, and Ser632 in IRS1 (Saini, 2010). Uncontrolled pro-inflammatory cytokine synthesis and secretion activate a pro-inflammatory signaling protein, such as TNF-α and JNK1 isoform 1, which are responsible for fatty tissue expansion and can induce hyperphosphorylation of IRS1 at Ser636 residue (Hotamisligil et al., 1996; Stuart et al., 2014). However, it is unknown, which specific serine residues or combinations of serine residues require hyperphosphorylation to evoke 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 (Stuart et al., 2014). Furthermore, serine hyperphosphorylation at Ser312 targets IRS1/2 for degradation, and diminish the IR-mediated signaling relay. These findings demonstrated the complexities of IRS proteins' roles and their relevance in modulating the insulin resistance. The role of inflammation in altered insulin signaling and insulin resistance is shown in Fig. 1.7.

1.5.2.3.2 Pro-inflammatory adipokine: Leptin (LEP)

Leptin (Lep) was discovered as the product of the obese (*ob*) gene (Zhang et al., 2005), located on chromosome 7q31.3 (Considine and Caro, 1997). Leptin is secreted chiefly from white adipose tissue, and its level is positively proportional to the amount of body fat, associated with food intake and appetite, energy homeostasis, basal metabolism, and insulin secretion (Friedman and Halaas, 1998; Mantzoros et al., 1999). Leptin, like other hormones, is secreted in pulsatile manner and exhibits considerable diurnal variation, with greater levels in the evening and early morning hours (Carvalheira et al., 2003; 2005). Circulating leptin levels largely reflect the amount of energy stored in fat and regulates acute changes in calorie intake (Carvalheira et al., 2003; Carvalheira et al., 2005).

LEP exerts its crucial physiological effect by binding to the ObR leptin receptor (LEPR). ObR is a single membrane-spanning receptor belonging to the class I cytokine receptor family, encoded by the *db* gene, located on chromosome 1p31. Alternative splicing or ectodomain shedding produces six ObR isoforms (LepRa-LepRf). These include one long form (LepRb; with an intracellular domain of 302 amino acids); four short forms (LepRa, LepRc, LepRd, and LepRf, with cytoplasmic tails of 30-40 amino acids with unique C-termini); and one soluble form (LepRe) (Mercer et al., 1996; Fei et al., 1997). LepRb is the most crucial and longest isoform with the capacity for strong signaling among these six as it contains three highly conserved tyrosine residues (Y985, Y1077, Y1138) required for efficient leptin signaling via the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) pathway (Nanjappa et al., 2011). Moreover, most of the biological functions of leptin are exerted by LepRb-JAK/STAT signaling cascade, which is prevalent in the hypothalamus, but also has a moderate presence in other tissues. LepRa and LepRc isoforms regulate the transportation of leptin across the blood-brain barrier (BBB) to the hypothalamus, and LepRf does the same to a lesser extent. LepRe isoform is a soluble binding protein that lacks transmembrane and cytoplasmic domains and plays an essential role in leptin transport across the blood-brain

barrier (BBB) and/or glomerular clearance (Hileman et al., 2000). The soluble LepRe isoform is directly secreted in mice, while in humans it is generated by ectodomain shedding and modulates the bio-availability of leptin (Ge et al., 2002; Wauman et al., 2011).

Leptin or leptin receptor deficiency not only causes excessive obesity (Montague et al., 1997), but also leads to abnormalities in hematopoiesis, immunity, reproduction, angiogenesis, bone formation, and blood pressure (Zhang et al., 2005). Furthermore, loss-of-function mutations in the *leptin* or *LEPR* genes or genetic ablation of leptin's central signaling result in severe, early-onset obesity (Chen et al., 1996; Clement et al., 1998; Montague et al., 1997). Several *LEP* and *LEPR* gene polymorphisms were studied in different populations for their potential association with serum leptin levels, obesity, T2D, and Metabolic syndrome (MetS) (Dasgupta et al., 2015; Sahin et al., 2013; Ghalandari et al., 2015). Among these variants, the *LEP* (5'UTR *rs2167270* G/A; G2548A *rs7799039* G/A) and *LEPR* (exon 6 Q223R *rs1137101* A/G; exon 14 K656N *rs1805094* G/C) single nucleotide polymorphisms (SNPs) have been studied in different populations (Ghalandari et al., 2015). There are only a few studies reported on these polymorphisms in the Indian population (Bains et al., 2020; Dar et al., 2019; Dasgupta et al., 2015; Murugesan et al., 2010) showing their association with obesity, BMI and T2D.

1.5.2.3.3 Leptin resistance

Leptin resistance is defined by the reduced effectiveness of circulating leptin to suppress appetite and weight gain and to promote energy expenditure, which is a primary risk factor for the development of obesity and other metabolic disorders (Liu et al., 2018). Defects in leptin transport across the BBB, impairment in the LepRb signaling and the defective hypothalamic neural circuitry that regulates energy homeostasis contribute to leptin resistance (Dey et al., 2021; Morris et al., 2009). Any lesion in the arcuate nucleus may also impair leptin signaling. The leptin resistance is commonly associated with hyperleptinemia. High leptin levels in the blood can promote overexpression of SOCS-3 and PTB1B, which contributes to leptin resistance via a feedback mechanism (Jamshidi et al., 2006). Leptin is transported across the BBB *via* LepRa and LepRe. Thus, improper binding of leptin to its receptors results in excess leptin in the bloodstream, which may also lead to leptin resistance (Dey et al., 2021; Morris et al., 2009). A recent study demonstrated that HFD-induced obesity leads to the activation of Matrix metalloproteinases-2 (MMP-2) in the hypothalamus, which impairs leptin signaling by ectodomain shedding of the LepRb and promoting leptin resistance. Further, knockout of this MMP-2 restored the expression of the leptin receptor and diminished leptin and insulin

resistance (Leal-Cerro et al., 2001).

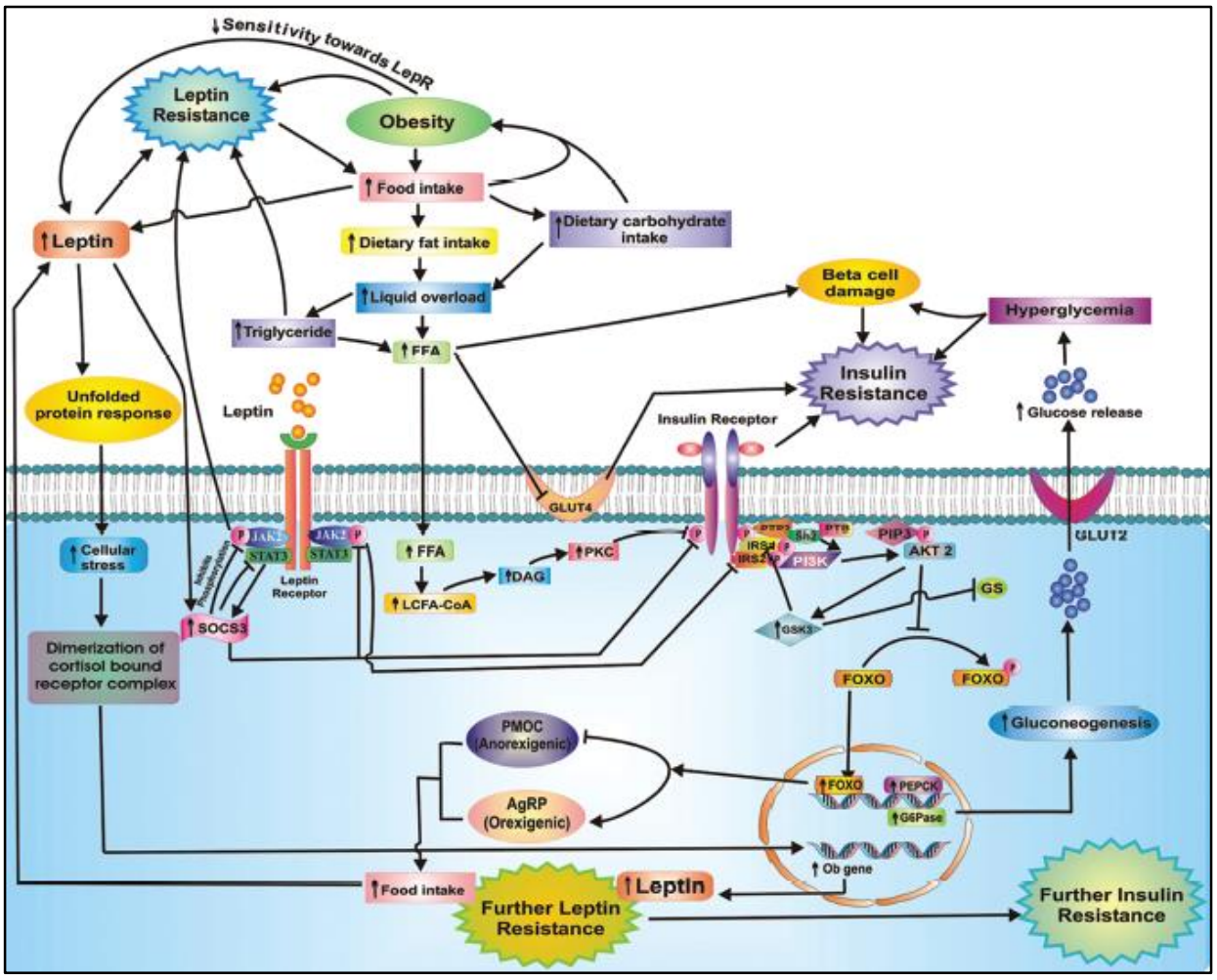

Figure 1.8. Mechanism of obesity and leptin resistance mediated insulin resistance. Leptin can modulate insulin signaling by different mechanisms. Inadequate leptin signaling to the hypothalamus results in overeating and leads to hyperglycemia and insulin resistance. Leptin and insulin resistance promote obesity. In obesity, adipose tissue secretes pro-inflammatory cytokines (IL-6, TNF-α), which can alter the gene expression of forkhead box O (FOXO) and SOCS3, resulting in leptin and insulin resistance. Increased SOCS3 levels inhibit the phosphorylation of insulin receptor substrate, and lead to insulin resistance (Dey et al., 2021).

Obesity and increased adiposity are commonly associated with elevated plasma FFA and leptin levels (Hardy et al., 2012), which reduces leptin effectiveness leading to hyperleptinemia and leptin resistance (Obradovic et al., 2021). The excess of cellular FFA increases intracellular long-chain fatty acyl CoA, which activates diacylglycerol (DAG) and protein kinase C (PKCs), which impaired the insulin receptor signaling and suppresses the expression of GLUT-4, which further promotes insulin resistance (Lee et al., 2006). Furthermore, the excess fat deposition promotes leptin synthesis and improper folding of signaling proteins, which activate unfolded protein response pathways, resulting in cellular stress and enhanced stress response. Leptin-

induces SOCS-3 expression and its activation inhibit Janus kinase 2 (JAK-2) and signal transducer and activator of transcription 3 (STAT-3) phosphorylation at the intracellular domain of the LEPR, and thereby it reduces leptin sensitivity and promotes leptin resistance (Knight et al., 2010).

While JAK2 of the LEPR-mediated PI3K activity is dependent on the activation of IRS2 of the INSR complex, insulin-stimulated PI3K activity is dependent on the activation of both IRS1 and IRS2 (Sims et al., 1968). The excess SOCS3-mediated dephosphorylation of JAK2 at LEPR during leptin resistance prevents IRS2 activation at INSR, thus resulting in insulin resistance (Stephens et al., 2015). Furthermore, SOCS3 directly inhibits tyrosine960 phosphorylation at INSR. Insulin, on the other hand, modulates the function of the forkhead transcription factor by inhibiting FOXO phosphorylation via Akt (Faouzi et al., 2007). The nuclear translocation of dephosphorylated FOXO induced by AKT results in an enhanced FOXO expression along with transcription of its target genes [i.e., Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase)], thereby leading to increased hepatic gluconeogenesis and hyperglycemia. Further, FOXO suppresses anorexigenic pro-opiomelanocortin (POMC) neurons, while stimulating orexigenic agouti-related protein/neuropeptide Y (AgRP/NPY) neurons, results in increased food intake and greater leptin resistance. In addition, insulin-induced AKT2 expression increases glycogen-synthase kinase-3 (GSK3) activity, which reduces glycogen synthase activity and prevents tyrosine phosphorylation of IRS1. Crosstalk between leptin and INSR signaling molecules, as well as leptin, FFA, SOCS3, JAK2, PKC, FOXO, and other signaling molecules, eventually amplifies the leptin resistance and consequent insulin resistance. Overall, these suggest that obesity and leptin resistance foster the insulin resistance (Fig. 1.8).

1.6 Management of Diabetes Mellitus (DM)

Despite the advances in DM management, the incidence and prevalence of this disease are increasing, causing many microvascular/macrovascular complications in the body, and leading to a shortened life expectancy and a worsened quality of life, as compared to healthy individuals (Srinivasan et al., 2008). Early-stage diagnosis and management of DM are crucial to prevent or halt its potential microvascular (neuropathy, nephropathy & retinopathy, and cardiomyopathy) and macrovascular (coronary artery disease, peripheral arterial disease, and stroke) complications (Forbes and Cooper, 2013).

1.6.1 Management of type 1 diabetes (T1D)

T1D is chronic condition characterized by the destruction of β-cells, leading to the eventual loss of insulin. Inadequate islet cell repair processes is another issue that compromises the glycemic control. Thus, insulin replacement therapy is the first-line treatment option for T1D, currently. The insulin-centric therapy predisposes a T1D patient to severe hypoglycemia episodes, lifelong reliance on exogenous insulin, insulin resistance, moderate obesity, and psychological disorders (Jacobson et al., 2013; Priya and Kalra, 2018). Such findings emphasize the need for transformations in the therapeutic approaches to restore glycemic control and complete insulin independence. The therapeutic usefulness of commercial insulin formulations has been improved by the use of self-monitoring devices for measuring blood glucose and HbA1c (Vora et al., 2015). The maintenance of normoglycemia without frequent hypoglycemic episodes remains a significant issue for the healthcare professionals, despite an effective introduction of several insulin delivery systems. Therefore, continuous insulin infusion devices for insulin administration have become popular in clinical practice in recent years, which allows for better HbA1c management with a lower hypoglycemic episode rate. Although the recent development of fast-acting and long-acting insulin analogues has improved T1D patients' quality of life, several obstacles are still remained.

Currently, a wide range of interventions, including artificial pancreas, immune modulation, sodium-glucose co-transporter-2 (SGLT2) inhibitor, glucagon-like peptide 1 (GLP-1) receptor agonist, amylin analogue, β-cell encapsulation, microencapsulation, stem cell therapies, incretin therapies, dipeptidyl peptidase-IV inhibitors (DPP-IV), and islet transplantation, are being used in clinical trials alongside the insulin therapy (Pathak et al., 2019; Warshauer et al., 2020). Primary islet transplantation offers promising treatment options for T1D patients. However, one of the most considerable barriers to the widespread adoption of islet transplantation is the scarcity of islets. Islet xenotransplantation has been investigated to address donor scarcity; however, ethical considerations and the genetic stability of transgenic islets remain challenging. Hence, treating T1D with insulin replacement therapy and human islet transplantation is practical and financially viable alternative. Many challenges impede the development of efficient human islets transplantation strategies, such as the substantial mortality of the islet cells in the post-transplantation period, which increase the number of islets required to establish glycemic control and insulin independence. For optimal treatment outcomes and total insulin independence, new strategies are essential. In line with these, for optimum clinical therapeutic effect, conventional T1D therapeutic modalities such as insulin replacement, SGLT2 inhibitors,

immunological therapies, and peptide agonists must be explored, either alone or in combination (Pathak et al., 2019).

1.6.2 Management of type 2 diabetes (T2D)

Major changes in the patient's lifestyle and pharmaceutical treatment approaches are critical for effective T2D management. These include changes in physical activity, dietary modifications, management of stress or associated factors, and improved sleeping patterns. The two primary factors affecting the energy balance are dietary intake and physical activity. A sufficient quantity of sleep (7 hours) is considered essential in treating T2D patients (Garber et al., 2016). Insomnia exacerbates insulin resistance, hypertension, hyperglycemia, and dyslipidemia (McNeil et al., 2013). Currently, different treatments (oral and injectable) are available for T2D management. For the majority of patients, metformin remains the primary line of therapy. Depending on each patient's traits, an alternative or second-line treatment is personalized.

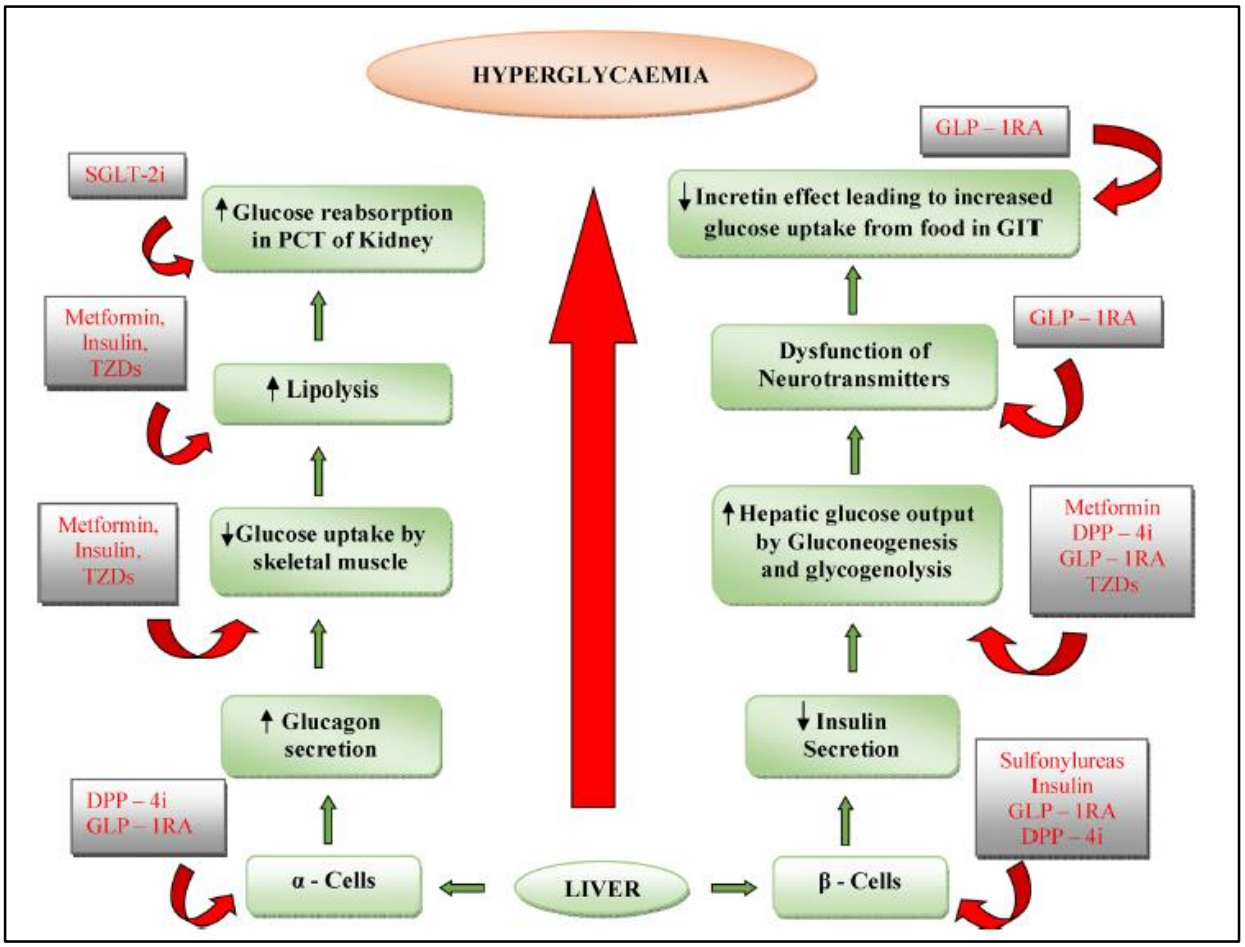

Fig. 1.9. Glucose metabolism and targeted therapies for T2D management (Padhi et al., 2020). [Abbreviations: TZDs: Thiazolidinediones, DPP4i: Dipeptidyl peptide-4 inhibitor, GLP-1RA: Glucagon-like peptide-1 receptor agonist, SGLT-2i: Sodium Glucose co-transporter 2 inhibitor]

The main conventional classes of oral drugs for the treatment of T2D comprise sulfonylureas/insulin secretagogues (increases insulin release from pancreatic islet, e.g., Glipizide, Glimepiride, Gliclazide, glibenclamide); biguanides (reduces hepatic glucose synthesis, e.g., metformin); Proliferator-Activated Receptor agonists (PPARs)/Insulin Sensitizers (increases insulin action, e.g., ciprofibrate, clofibrate, bezafibrate, and fenofibrate); Thiazolidinediones/Insulin Sensitizers (increases insulin action, e.g., rosiglitazone and pioglitazone); α-glucosidase inhibitors (interferes with glucose absorption in the gut, e.g., acarbose, miglitol and voglibose); Amylin analogues (delays the gastric emptying and suppresses the secretion of glucagon, e.g., Symlin); Incretin mimetics/DPP-IV inhibitors (increases the activity of GLP-1,e.g., sitagliptin, saxagliptin, linagliptin, alogliptin, Anagliptin, vildagliptin); SGLT2 antagonists/ inhibitors (prevents reabsorption of glucose and enhances the excretion of glucose, e.g., dapagliflozin, canagliflozin, empagliflozin, ipragliflozin, and tofogliflozin); Injectable drugs include glucagon-like peptide 1 receptor agonists/Incretin mimetics (GLP-1 RA) (increases insulin secretion and reduce glucagon secretion, e.g., exenatide, lixisenatide, liraglutide, albiglutide, dulaglutide) and insulin (Pramanik et al., 2018; Padhi et al., 2020). Further, the characteristics and side effects of the most widely used antidiabetic drugs are shown in Table 1.2 (Lipscombe et al., 2018; Maruthur et al 2016; Mearns et al 2015; Liu et al 2012. Sherifali et al 2010).

Table 1.2. Characteristics and side effects of the most widely used antidiabetic drugs.

Class	**Drug**	**Risk of Hypoglycemia**	**Effect on Weight**	**Side effects/Other therapeutic considerations**
First Line Biguanide	Metformin	Negligible as monotherapy	Neutral	GI side effects Vitamin B12 deficiency Hepatic failure
Second Line Incretin	**DPP-4 inhibitors** Alogliptin Linagliptin Saxagliptin Sitagliptin	Negligible as monotherapy	Neutral	Rare cases of pancreatitis Rare cases of severe joint pain
	GLP-1 receptor agonists *Short-acting* Exenatide Lixisenatide *Longer-acting*	Negligible as monotherapy	1.6 to 3 kg loss	Nausea, vomiting, diarrhea. Rare cases of acute gallstone disease

	Dulaglutide Liraglutide			
SGLT-2 inhibitors	Canagliflozin Dapagliflozin Empagliflozin	Negligible as monotherapy	2 to 3 kg loss	Genital mycotic infections Urinary tract infections Hypotension Slight increase in LDL-C Increased risk of fractures (canagliflozin) Acute kidney injury (canagliflozin & dapagliflozin)
Alpha-glucosidase inhibitor	Acarbose	Negligible as monotherapy	Neutral	GI side effects
Thiazolidine dione (TZD)	**Meglitinides** Repaglinide Pioglitazone Rosiglitazone	Negligible as monotherapy	2.5 to 5 kg gain	May induce edema and/or macular edema May induce congestive heart failure Higher occurrence of fractures Slight increase in HDL-C
Weight loss agent	Orlistat	Negligible as monotherapy	3 to 4 kg loss	Diarrhea and other GI side effects
Insulin secretagogue	**Sulfonylureas** Gliclazide Glimepiride Glyburide	Moderate risk	1.5 to 2.5 kg gain	Rapid FBG lowering response Lower risk of CV events, hypoglycemia, and mortality.
Insulin	**Bolus (prandial) Insulins** *Rapid-acting analogues* Aspart Glulisine Lispro U-100 Lispro U-200 *Short-acting* Regular **Basal Insulins** *Intermediate-acting* NPH *Long-acting analogues* Degludec U-100 Degludec U-200 Detemir Glargine U-100 Glargine U-300 **Premixed**	Significant risk	4 to 5 kg gain 0 to 0.4 kg gain (long-acting analogue)	Hypoglycemic episodes

	Insulins Premixed regular-NPH Biphasic insulin aspart			

[Abbreviations: A1C, glycated hemoglobin; BG, blood glucose; CrCl, creatinine clearance; CV, cardiovascular; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GI, gastrointestinal; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.]

These medications are either taken as monotherapy or in combination with other hypoglycemics. The American Association of Clinical Endocrinologists (AACE) treatment algorithm (Garber et al., 2017) recommended that patients with an HbA1c level of >7.5% should receive combination therapy with metformin plus an additional drug. Cai et al. (2014) asked the question of whether initial combination therapy is more efficacious than monotherapy in terms of glucose control and confirmed safety. They found that all initial combination therapies resulted in significant HbA1c reductions compared to monotherapy. Intriguingly, compared to metformin monotherapy, the initial combination therapies of DPP-4 inhibitors plus metformin and SGLT2 inhibitors plus metformin exhibited similar risks of hypoglycemia, but the initial combination therapies of sulfonylurea plus metformin and thiazolidinedione plus metformin exhibited higher risks of hypoglycemia (Cai et al., 2014) (Table 1.3). Despite several treatment options available for the management of T2D, major challenges in efficient diabetes treatment include: i) optimizing the existing therapies to guarantee optimum and balanced glucose concentrations and reducing long-term diabetes-related complications, and ii) the most patients experience serious or minor adverse effects, such as hypoglycemic episodes, gastrointestinal discomfort, fatigue, weight loss, as well as liver and kidney complications (Tan et al., 2019). As current treatments only help to attenuate hyperglycemia and other symptomatic traits, recent research is focusing on developing novel drugs with the least side effects. However, complete cure remains ambiguous, understanding the potential modalities for β-cell preservation and regeneration-based therapies is critical for managing T1D and T2D.

Table 1.3. Comparisons of initial combination therapy *versus* monotherapy with respect to the risks of hypoglycemia and other adverse effects.

Comparison group	**No. of patients**	**Relative risk**	***P* value**

DPP-4 inhibitors + metformin vs. DPP-4 inhibitors			
Adverse effect	1967/1951	1.07	0.29
Drug-related adverse effect	1514/1489	1.73	< 0.001
Hypoglycemia	1824/1823	1.84	0.007
Gastrointestinal-related adverse effects	1584/1591	2.19	< 0.001
Serious adverse effect	1742/1746	0.70	0.11
Discontinuation due to adverse effect	1584/1591	0.77	0.29
DPP-4 inhibitors + metformin vs. metformin			
Adverse effect	3379/3375	0.92	0.09
Drug-related adverse effect	2926/2920	0.97	0.63
Hypoglycemia	3379/3375	1.15	0.38
Gastrointestinal-related adverse effects	2996/2989	0.91	0.17
Serious adverse effect	3154/3150	0.71	0.05
Discontinuation due to adverse effect	2996/2989	0.88	0.44
SGLT2 inhibitors + metformin vs. metformin			
Adverse effect	978/974	1.19	0.06
Drug-related adverse effect	978/974	1.45	0.004
Hypoglycemia	642/646	1.37	0.42
Gastrointestinal-related adverse effects	978/974	0.72	0.25
Serious adverse effect	978/974	0.84	0.62
Discontinuation due to adverse effect	978/974	0.82	0.46
SGLT2 inhibitors + metformin vs. SGLT2 inhibitors			
Adverse effect	1220/1236	1.16	0.07
Drug-related adverse effect	1220/1236	1.13	0.31
Hypoglycemia	642/646	2.23	0.02
Gastrointestinal-related adverse effects	978/989	1.99	0.002
Serious adverse effect	978/989	0.62	0.13
Discontinuation due to adverse effect	978/989	0.83	0.50
DPP-4 inhibitors + TZD vs. TZD			
Adverse effect	1154/1138	0.94	0.50
Drug-related adverse effect	1265/1107	1.06	0.70
Hypoglycemia	1413/1268	1.08	0.65
Gastrointestinal-related adverse effects	1265/1107	0.86	0.50
Serious adverse effect	1170/1140	1.31	0.22
Discontinuation due to adverse effect	1006/977	0.80	0.42
DPP-4 inhibitors + TZD vs. DPP-4 inhibitors			
Adverse effect	502/504	1.09	0.50
Drug-related adverse effect	350/354	1.40	0.12
Hypoglycemia	350/354	0.84	0.57
Gastrointestinal-related adverse effects	-	-	-
Serious adverse effect	350/354	1.31	0.44
Discontinuation due to adverse effect	-	-	-
SU/glinide + metformin vs. metformin			
Adverse effect	425/429	1.26	0.17
Hypoglycemia	425/429	8.91	0.02
Gastrointestinal-related adverse effects	425/429	0.70	0.06
Serious adverse effect	-	-	-
Discontinuation due to adverse effect	-	-	-

SU/glinide + metformin vs. SU/glinide			
Adverse effect	425/416	0.98	0.92
Hypoglycemia	425/416	0.63	\0.001
Gastrointestinal-related adverse effects	425/416	1.42	0.01
Serious adverse effect	-	-	-
Discontinuation due to adverse effect	-	-	-
TZD + metformin vs. metformin			
Adverse effect	954/970	1.06	0.55
Hypoglycemia	954/970	1.60	0.03
Gastrointestinal-related adverse effects	954/970	0.87	0.07
Serious adverse effect	954/970	0.98	0.91
Discontinuation due to adverse effect	954/970	1.06	0.76
TZD + metformin vs. TZD			
Adverse effect	356/348	1.31	0.08
Hypoglycemia	356/348	1.53	0.20
Gastrointestinal-related adverse effects	-	-	-
Serious adverse effect	356/348	0.87	0.79
Discontinuation due to adverse effect	-	-	-

[Abbreviations: DPP-4: Dipeptidyl peptide-4; SGLT-2: Sodium Glucose co-transporter 2; SU: Sulfonylurea; TZD: Thiazolidinedione.]

1.7 β-cell regeneration

In response to metabolic demands during pregnancy and obesity, pancreatic tissue can maintain or expand its β-cell mass. Since the loss of functional β-cell mass is the hallmark of DM, it is pertinent to understand the pancreatic regeneration in the context of this disease. Existing DM treatments do not reverse or control the disease. Therefore, developing regenerative therapies that prevent or even reverse the deterioration of β-cell function endogenously is essential. β-cell regeneration can be accomplished by: β-cell proliferation, β-cell neogenesis, and β-cell transdifferentiation; these are the most direct, simple, and least invasive ways to increase the β-cell mass.

1.7.1 β-cell proliferation

Proliferation is the expansion of pre-existing adult β-cells through cell division, which is an a desirable approach for generating new β-cells for therapeutic purposes During the development of the mouse and human pancreas, β-cells are able to replicate readily in the embryonic and neonatal stages. After the embryonic and neonatal stages, the ability of β-cells to replicate rapidly decreases. However, this replication can be stimulated by various metabolic stressors like in pregnancy and obesity (Baeyens et al., 2016; Cox et al., 2016). The rate of β-cell proliferation decreases rapidly in the early stages of life, and in adults, the rate of

β-cell division is very low, resulting in the fixation of β-cell mass during adolescence (Meier et al., 2008; Perl et al., 2010). So far, identifying molecules that may trigger adult β-cell replication has been challenging due to: i) species-dependent molecular differences between mouse and human β-cells, and ii) the factors that can stimulate replication of mouse β-cells (which do not offer similar activities in human β-cells) (Aamodt et al., 2016; Karakose et al., 2018). Another hurdle is that adult β-cells are resistant to mitogens that can induce proliferation in juvenile β-cells from young donors (Dai et al., 2017). Adult β-cells are less susceptible to proliferation than juvenile β-cells, as they express more cell cycles inhibitors, such as p16INK4a, and fewer cell cycles activators, such as FoxM1, cyclins, and cyclin-dependent kinases (Fiaschi-Taesch et al., 2013; Golson et al., 2015; Tschen et al., 2017).

The hunt for factors that can trigger adult β-cell replication has become more complex with the discovery of β-cell heterogeneity. Although several subpopulations of β-cells exist within an islet, it is unknown whether all β-cells can undergo proliferation (Benninger and Hodson, 2018). It has been demonstrated that Flattop (Fltp), an effector of the Wnt/planar cell polarity signaling pathway, marks a subset of mature β-cells with greater functionality, but with lower proliferative potential (Bader et al., 2016). Identifying markers that distinguish β-cells with a higher proliferative capacity would potentially allow researchers to target this specific population. However, it has been observed that β-cell proliferation often occurs at the cost of insulin secretion, and replicating β-cells bear a greater resemblance to immature β-cells. For instance, when replication in adult mouse β-cells was promoted by exogenously expressing c-Myc, these β-cells showed decreased expression of genes which are crucial for glucose sensing and insulin secretion [e.g., glucose transporter 2 (GLUT2), & proprotein convertase 1/3 (PC1/3)], and a transcriptional marker of mature β-cells [e.g., pancreatic and duodenal homeobox 1 (PDX1), mast cell function-associated antigen (MAFA), & homeodomain protein NK2 homeobox 2 (NKX2.2)] (Puri et al., 2018). Thus, the balance between proliferation and functionality must also be considered when identifying new drugs/ molecules to expand the β-cell mass.

1.7.2 β-cell neogenesis

Neogenesis is defined as *de novo* islet formation from pancreatic progenitor cells. An endocrine progenitor population that resides within the pancreatic ductal epithelium, identified by the transcription factor Neurogenin3 (Ngn3), gives rise to pancreatic β-cells during embryonic development. $Ngn3^{+}$ endocrine progenitor cells in mice and humans differentiate into all four

adult endocrine cell types throughout the embryogenesis, although their numbers decrease after birth (Gradwohl et al., 2000; Gu et al., 2002). Ngn3 null mice lack all islet endocrine cells, demonstrating that Ngn3 is crucial for endocrine neogenesis during development (Gradwohl et al., 2000). Many researchers have investigated whether the embryonic endocrine differentiation programme may be re-activated in adult pancreatic ducts to serve as a possible source of new β-cells, since endocrine cells arise from the ductal epithelium during the development. However, whether this happens endogenously or under specific pathological situations is debatable. Interestingly, studies using pancreatic injury models, such as pancreatic duct ligation or partial pancreatectomy, demonstrated the reappearance of $Ngn3^{+}$ progenitor cells within the adult ductal epithelium with tiny clusters of endocrine cells near these ducts, indicating that neogenesis can occur (Xu et al., 2008; Van de Casteele et al., 2013). However, investigations using comparable methods suggested that the neogenesis did not occur, indicating that this process is difficult to trigger or uncommon (Cavelti-Weder et al., 2013; Menge et al., 2008). Lineage tracing of the ductal lineage with an inducible Cre recombinase (CreER) controlled by a chunk of the human carbonic anhydrase promoter demonstrated that endocrine cells could arise from mature ducts, but tests with Hnf1CreER and Sox9CreER demonstrated the conflicting results (Inada et al., 2008; Kopp et al., 2011). Moreover, obtaining the confirmation of β-cell neogenesis in humans has proven to be difficult. Based on the finding of islet cell clusters near or closely opposed to ducts in donor pancreata, potential evidence of ductal-derived β-cells has been proposed (Dirice et al., 2019; Butler et al., 2003). Ductal cells positive for immature cell markers have also been found in samples from pregnant women and T2D patients, and their numbers appear to rise in obese people. Furthermore, human ductal cells can be induced to express the pancreatic markers and insulin in *ex-vivo* culture systems (Suarez-Pinzon et al., 2005; Yatoh et al., 2007). However, it is challenging to prove that human β-cell neogenesis occurs considerably *in-vivo,* without the ability to trace the genetic lineage of human ductal cells.

1.7.3 β-cell transdifferentiation

Transdifferentiation defines the conversion of one differentiated cell type into another cell type. While it was previously believed that endocrine cells were a stable and fully differentiated population, studies have demonstrated that they are highly flexible in response to stress or genetic modification (Swisa et al., 2017). Genetic studies revealed that ectopic expression of Pax4 or Arx inactivation in α-cells resulted in the neogenesis of functional β-cells from α-cells (Ben-Othman et al., 2017; Courtney et al., 2013). Similarly, inactivating the tumor suppressor

Men1 in α-cells resulted in their conversion to β-cells, but with the production of insulinomas (Lu et al., 2010). This was further demonstrated after severe β-cell ablation using the diphtheria toxin receptor (DTR) approach. The transdifferentiated α-cells accounted for a high fraction of newly produced β-cells when largely eliminated in post-pubescent mice. Pre-pubescent mice lack this transdifferentiation potential; however, δ-cells can transdifferentiate into β-cells in a Foxo1-dependent way. Both the procedures resulted in bi-hormonal cells (expressing insulin and glucagon), suggesting a mechanism of the gradual conversion (Chera et al., 2014, Thorel et al., 2010). The simultaneous silencing of Arx and DNA methyltransferase 1 (Dnmt1) enhanced this conversion in mice. Intriguingly, coupled deletion of these genes and expression of β-cell markers was detected in α-cells of T1D human islets, indicating that transdifferentiation may be used for β-cell mass replenishment in humans (Chakravarthy et al., 2017). Moreover, following the ectopic expression of PDX1 and MAFA, *in-vitro* re-aggregated adult human α- or PP- cells obtained some aspects of β-cell physiology, including the insulin production and the glucose sensing (Furuyama et al., 2019). However, pharmacological stimulation of α-to-β cell conversion would provide a possible means of the β-cell mass restoration in diabetes.

Several studies have demonstrated that use of adenoviral transduction of one or a combination of pancreatic transcription factors, (such as PDX1, neuronal differentiation 1 (NeuroD1), or a combination of PDX1, NGN3, and MAFA, which are referred to as PNM factors), can induce insulin-positive cells *in-vivo* in other tissues such as liver, kidney, and gut (Banga et al., 2012; Ferber et al., 2000; Kojima et al., 2003). Terminally differentiated exocrine tissue within the pancreas has also been proposed as a source of *de novo* endocrine cells. Zhou et al. (2008) have demonstrated that delivering PNM factors through an adenoviral vector into the pancreas of an adult immune-compromised mouse could transform acinar cells into β-cells. However, the inherent ability of pancreatic exocrine tissue to produce β-cells without adenoviral injection was not elucidated (Clayton et al., 2016; Desai et al., 2007). Several studies have demonstrated that reprogramming may take place throughout a mouse's lifespan in response to physiological cues like several rounds of pregnancy and also after the β-cell injury and that δ-cells can convert to β-cells in young mice (Chera et al., 2014; Ye et al., 2016). The subject of whether endocrine cells may transdifferentiate into β-cells in patients with pancreatic disease is still open, due to the incapability to conduct lineage tracing experiments in humans. Furthermore, studies demonstrated that it is possible to generate β-cells in vitro through transdifferentiation of non-pancreatic pluripotent progenitors such as human pluripotent stem cells (hPSCs) (Rosler et al., 2004) and induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006).

When implanted into diabetic rodents, these newly generated β-cells were able to release insulin (Kroon et al., 2008; Rezania et al., 2014; Velazco-Cruz et al., 2019). Despite several recent scientific discoveries, it is still unknown, if human pancreatic β-cells have inherent regeneration ability. However, existing data imply that β-cells may be capable of regeneration under certain conditions (Fig. 1.10).

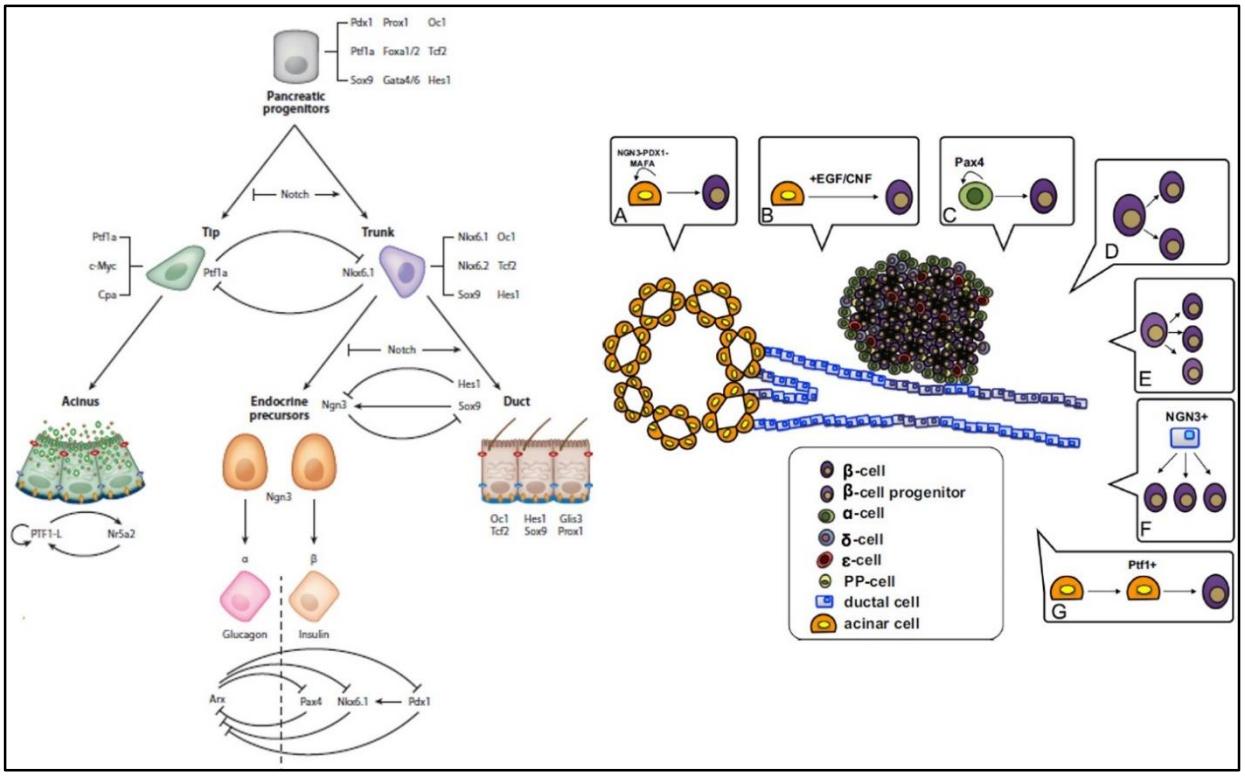

Fig. 1.10. Lineage decisions during pancreas development. An illustration of the crucial transcription factors to the commitment and specification of several pancreatic lineages (Shih et al., 2013). **Potential strategies for regenerating β-cells:** (A) Induction of new β-like cells by ectopic expression of NGN3, PDX1, and MAFA in acinar cells; (B) Acinar cells transform into β-like cells in response to epidermal growth factor (EGF) and ciliary neurotrophic factor (CNF) treatment; (C) Ectopic PAX4 expression in α-cells promotes the differentiation of these cells into β-cells; (D) The proliferation of pre-existing β-cells; (E) Intra-islet multipotent pancreatic progenitors regenerate β-cells; (F) β-cells neogenesis from $NGN3^{+}$ ductal progenitors; and (G) Regeneration of β-cell from Ptf1+ acinar endocrine progenitors (Migliorini et al., 2014).

1.7.4 Role of biomolecules in β-cell regeneration

Biomolecules are active agonists produced in the biological systems. These compounds have tissue-specific effects on different cell types as well as, autocrine and paracrine actions. This might explain their capacity to regenerate in both intra- and extra-pancreatic environments. It is essential to regenerate damaged tissue by promoting the regeneration of competent cells. Hence, the use of potent biomolecules is an appealing alternative for the β-cell regeneration.

Several strategies have been used to identify drugs, growth factors, and biomolecules, which are capable to induce human β-cell regeneration. Unbiased screening and candidate approaches to β-cell regenerative drug discovery have suggested that gamma-aminobutyric acid (GABA), glucagon-like peptide-1 (GLP-1), cholecystokinin octapeptide (CCK-8), dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) inhibitors, prolactin/placental lactogen (PRL/PL), osteoprotegerin/denosumab, inhibitors of the receptor activator of nuclear kappa-B ligand (RANKL), the TGF-β superfamily, serpin B1, β-cellulin, V-growth factor (VGF)-derived peptide called TLQP-21 and cell cycle inhibitors (p18, p21) may have mitogenic effects on β-cells (Karakose et al., 2018; Rathwa et al., 2020).

1.8 Melatonin

Lifestyle changes, including a tendency of nocturnality and consuming high-calorie foods, disrupt the natural sleep/wake cycle and circadian rhythms, increasing the likelihood of developing diabetes. (Scheer et al., 2009). Melatonin (N-acetyl-5-methoxytryptamine), a small indolic molecule generated from tryptophan, is chiefly secreted by the pineal gland and is found locally in various other tissues (Reiter et al., 1991). Melatonin is regarded as the "hormone of darkness," a circadian rhythm regulator with pleiotropic effects, and is also functionally linked to glucose metabolism (Mulder et al., 2009). In mammals, plasma melatonin levels are reported to be low (10–20 pg/mL) during the day, and high (80–100 pg/mL) at night (Simonneaux and Ribelayga, 2003). The active absorption of the amino acid tryptophan into the pineal gland initiates the melatonin production. Tryptophan is hydroxylated and decarboxylated to form serotonin (5-hydroxytryptamine, 5-HT). Serotonin N-acetyltransferase (or Arylalkylamine N acetyltransferase, AANAT) acetylates 5-HT to produce N-acetylserotonin (NAS), which is then converted to melatonin (N-acetyl-5-methoxytryptamine) by the enzyme hydroxyindole-O-methyltransferase (HIOMT) (Axelrod and Weissbach, 1960). Peschke et al. (2013) found that there could be a link between melatonin and T2D, as insulin secretion decreases as the concentration of melatonin in plasma increases. In addition, pinealectomy leads to loss of melatonin, which leads to an increase in insulin levels due to the absence of melatonin's inhibitory effect on insulin secretion (Nishida et al., 2003).

Melatonin exerts its pleiotropic effects through two receptors, namely MT1 (MTNR1A) and MT2 (MTNR1B), which are expressed in pancreatic islets (including α, β, and δ cells) as well as in insulin-sensitive tissues, apart from several other tissues (Ramracheya et al., 2008). In pancreatic β-cell, intracellular signal transduction pathways are regulated by melatonin via MT1- and

MT2-membrane receptors, which comprise cAMP-, cGMP-, and IP3-signaling pathways, as shown in Fig. 1.11. Melatonin suppresses insulin release induced by cAMP or cGMP *via* Gi protein-coupled MT1 and MT2 receptors. Melatonin, on the other hand, induces IP3-mediated Ca^{2+} flow into the cell from intracellular stores (Bach et al., 2005), which in turn triggers insulin release by pancreatic β-cell. Melatonin's MT2 receptor-dependent signaling pathway activates phospholipase C (PLC) via Gq proteins, thereby significantly increasing the inositol triphosphate (IP3)/Ca2+ from intracellular reserves (Peschke et al., 2006). Furthermore, Diacylglycerol (DAG) is produced as a result of the activity of phospholipase C (PLC), and it can activate protein kinase D (PKD) and protein kinase C (PKC) through the MAPK p38 pathway, and enhance the insulin vesicle fusion.

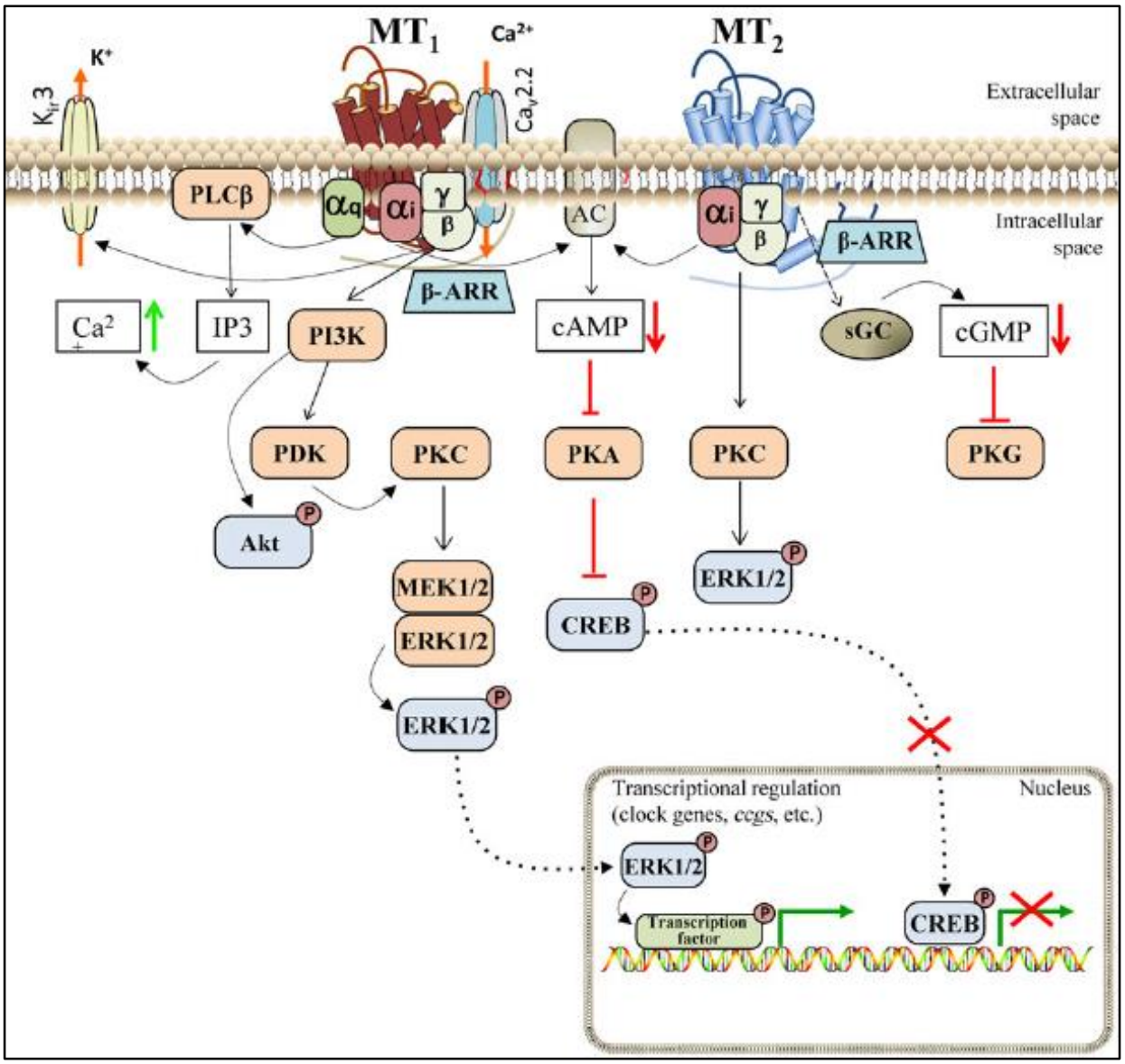

Fig. 1.11. Activation of intracellular signaling pathways by melatonin receptors. MT1 receptor activation induces Gαi activation, which decreases the levels of cAMP and leads to

Gβγ-dependent activation of the PI3K/Akt, PKC, and ERK pathways. The interaction of MT1 with Gq leads to PLC activation and an increase in intracellular Ca^{2+}. Cav2.2-mediated calcium entry *via* Gβγ-subunits is tonically inhibited by the physical contact of MT1 receptors with Cav2.2 channels. Activation of MT2 receptors trigger Gαi-dependent cAMP and ERK signaling pathways and inhibit cGMP levels. Melatonin induces β-arrestin recruitment to both MT1 and MT2 receptors, but β-arrestin-dependent down-streaming signaling is not yet reported (Cecon et al., 2018). [Abbreviations: PIP2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; DAG, diacylglycerol; β-ARR, β-arrestin; Cav2.2, voltage-gated calcium channel; ccgs, clock-controlled genes; CREB, cAMP-responsive element binding; Kir3, G protein-coupled inwardly rectifying potassium channel; ERK, extracellular signal-related kinase]

Melatonin is essential for insulin-stimulated phosphatidylinositol 3-kinase (PI3K)-AKT activity in mouse liver, and it suppresses the hepatic glucose production, and activates glycogen synthesis in the human hepatocyte cell line (HepG2), possibly *via* a PKC-AKT-glycogen synthase kinase-3 (GSK3) pathway. Melatonin increases the rate of glucose absorption in mouse skeletal muscle by activating the insulin receptor substrate 1 (IRS1)-PI3K-PKC pathway. In the PAZ6 human brown adipocyte cell line, melatonin acutely inhibits cGMP production and reduces glucose uptake and GLUT4 expression with prolonged treatment (Karamitri and Jockers, 2019). Melatonin is a naturally occurring substance that scavenges free radicals, modulates Nrf2 (Ahmadi and Ashrafizadeh, 2020), and possesses anti-inflammatory and antioxidant properties (Hacışevki and Baba, 2018). Melatonin signaling has been shown to enhance insulin secretion and β-cell survival. This effect may be due to a decrease in β-cell apoptosis and oxidative stress in human islets exposed to chronic hyperglycemia, as well as in islets from patients with type 2 diabetes (Costes et al., 2015). Moreover, Studies conducted on rodents have indicated that melatonin can decrease body fat and HbA1c levels, while also increasing insulin sensitivity and GLUT4 expression in peripheral tissues of a diet-induced obese T2D mouse model (Karamitri and Jockers, 2019; Patel, 2021). Since DM is more prevalent in individuals aged 40-60 years and older, and fact that melatonin levels decrease with age, there should be more preclinical trials conducted using various experimental diabetes models and clinical trials involving humans to evaluate melatonin's potential as a therapeutic agent for diabetes. Further, the effects of melatonin on metabolic profile/insulin sensitivity in pre-clinical as well as in patients is summarized in table 1.4.

Table 1.4. Therapeutic effects of melatonin on metabolic profile in diabetes model.

Model	Strain/ Cell line	Dose of Melatonin and way of	Treatment period	Effects	References

		administration			
HFD-induced (12 weeks)/ cell culture	Male C57BL/6J mouse/ HepG2 cell line	10 mg/kg ip/ 1nM	2 weeks/ various time intervals	Improved glucose tolerance, insulin tolerance/glycogen synthesis.	Faria et al., 2013
STZ-induced (120 mg/kg ip)	Male Wistar rat	1 mg/kg ip	8 weeks	Increased adiponectin and fructosamine. Improved oxidative stress conditions.	Thomas et al., 2016
HFD- induced (10 weeks)	Male Wistar rats	25 µg/mL in drinking water	10 weeks	Decreased TG, TC, LDL, uric acid. Increased HDL and insulin sensitivity.	Cano Barquilla et al., 2014
High calorie diet induced obesity model (16 weeks)	Male Wistar rat	4 mg/kg in drinking water	8 weeks	Reduced body weight, visceral adiposity, TG, HOMA-IR index.	Nduhiraban di et al., 2011
STZ-induced (70 mg/kg ip) T1D	Male Wistar rats	Daily melatonin of 200 µg/kg/day ip three days prior to induction of Diabetes	4 weeks	Insignificant effect on plasma glucose levels, serum ALT and AST levels.	El-Batch et al., 2011
HFD-induced T2D	Male Wistar rats	1 or 10 mg/kg/day ip	2 weeks	Decreased abdominal fat weight but no change in body weight.	Kitagawa et al., 2012
Circadian-rhythm disruption and HFD- induced (12 weeks)	Male Sprague-Dawley rats	20 mg/kg po	12 weeks	Improved insulin sensitivity, adiposity, circadian rhythm, reduced β-cell apoptosis.	Agil et al., 2011
HFD (45 days), STZ (40 mg/kg), Nicotinamide (200 mg/kg) – induced T2D	Male Sprague-Dawley rats	5 mg/kg po twice a day along with exercise (swimming of 40 min/ day for 5 days/week)	6 weeks	Amelioration of increased body weight non-invasive blood pressure, dyslipidemia, increased pro-inflammatory cytokines.	Rahman et al., 2017
Genetically obese model (fa/fa, fa, -)	Male Zucker diabetic fatty rats	10 mg/kg in drinking water	6 weeks	Reduced fasting hyperglycemia, insulinemia, hyperleptinemia, serum FFA levels and adiponectin levels.	Agil et al., 2012
Nicotinamide (120 mg/kg ip) / STZ induced (50 mg/kg ip)	Male ICR mice	10,20,50 mg/kg ip	6 weeks	Increased adiponectin levels and decreased leptin levels.	Lo et al., 2017
High-fat diet-induced T2D model	Male Wistar rats	25 µg/mL in drinking water	10 weeks	Decreased circulating levels of IL-1β, IL-6, TNF-α, IFN-γ, CRP and restored the IL-4 and IL-10 levels.	Cano-Barquilla et al., 2014

Zucker diabetic fatty rat model	Zucker rat	10 g/kg ip	6 weeks	Decreased IL-6, TNF-α and CRP.	Agil et al., 2013
Female T1D patients	-	10 mg daily	1 week	Lowers blood pressure	Cavallo et al., 2004
T2D patients	-	10 mg daily	12 weeks	Improved dyslipidemia	Kadhim et al., 2006
T2D patients	-	6 mg daily	13 months	Reduced HbA1c, FBG and HDL levels. No changes observed in elevated TG, TC and LDL levels.	Rezvanfar et al., 2017
Patients with obesity	-	10 mg daily	1 month	Reduced body weight	Mesri Alamdari et al., 2015
Healthy obese women	-	6 mg daily	40 days	No effect on body weight	Szewczyk-Golec et al., 2017

[Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FBG: Fasting blood glucose; FFA: Free fatty acid; HDL: High density lipoprotein; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL: Low density lipoprotein; TC: Total cholesterol; TG: Triglycerides.]

1.9 γ-Aminobutyric acid (GABA)

γ-Amino butyric acid (GABA), a major neurotransmitter, is also produced by peripheral non-neuronal tissues and has astonishing physiological effects in DM due to its anti-diabetic, antioxidant, anti-inflammatory, and immunomodulatory properties (Rashmi et al., 2018). Recently, GABA has been identified as a molecule that has crucial beneficial effects, such as: i) stimulating endogenous β-cell proliferation and suppressing detrimental immune reactions and consequent apoptosis, and ii) ameliorating metabolic profile by improving peripheral insulin sensitivity, and iii) having a crucial role in islet-cell hormone homeostasis (Soltani et al., 2011; Prud'homme et al., 2013; Purwana et al., 2014; Wang et al., 2019; Rathwa et al., 2022). L-glutamate is decarboxylated to GABA by glutamate decarboxylase isoforms (GAD 65/GAD 67). In neurons, GAD65 is predominantly present in synaptic vesicles, whereas GAD67 has a cytosolic location (Rathwa et al., 2020; Wang et al., 2019). Pancreatic expression of GAD differs between human and mouse β-cells. In humans, GAD65 is predominant, while in mice GAD67 is predominant (Wang et al., 2019). GABA exerts different kinds of responses in α- and β-cells, which include several excitatory and inhibitory actions on β-cells and α-cells (Braun et al. 2010). GABA exerts its biological effects by activating GABA receptors ($GABA_AR$ and $GABA_BR$) that are expressed in a variety of peripheral tissues, including

pancreatic islet cells and immune cells, such as T and B lymphocytes (Tian et al., 2004). $GABA_AR$ are heteropentamers of various subunits that create fast-acting chloride channels (Rudolph and Knoflach 2011). Until present, 19 $GABA_AR$ subunits (α 1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3) have been identified (Olsen and Sieghart 2009). $GABA_ARs$ with various subunit combinations possess different pharmacological properties. In human β-cells, it usually contains a combination of several α chains, β3, and γ2 subunits (e.g., α2β3γ2, α5β3γ2, α2α5β3γ2), and the variation occurs as a result of the difference in the α-chain subunit (Korol et al., 2018). GABA acts *via* $GABA_AR$ on the α-cells and suppresses α-cell exocytosis and glucagon release due to membrane hyperpolarization and deactivation of the voltage gated calcium channel (VGCC). In contrast, GABA exerts stimulatory effects on β-cell insulin secretion via membrane depolarization and a VGCC-dependent Ca^{2+} movement via $GABA_AR$ (Wang et al., 2019). $GABA_ARs$ have been found in α, β, and δ cells of human islets (Braun et al., 2010) and α- (Bailey et al., 2007) and β-cells in murine islets (Soltani et al., 2011). In contrast, $GABA_BRs$ is a metabotropic G-protein-coupled receptor (GPCR) that functions as a secondary messenger in GABAergic signaling (Cryan and Kaupmann, 2005), and is composed of two invariable subunits: B1 and B2 (White et al., 1998). This slow-acting receptor is linked to K^+ channels. Activation of $GABA_BR$ stimulates the opening of K^+ channels, resulting in membrane hyperpolarization and a decrease in adenylyl cyclase activity (Hashimoto and Kuriyama, 1997). $GABA_BRs$ have been found in humans (Braun et al., 2010) and mouse β-cells (Brice et al. 2002), and rat α- and β-cells (Braun et al., 2004). GABA-induced β-cell membrane depolarization and Ca^{2+} influx can activate the PI3K/Akt signaling cascade, which plays a pivotal role in β-cell protection and survival (Wang et al., 2004; Soltani et al., 2011; Purwana et al., 2014). GABA promotes the phosphorylation of CREB (cAMP response element-binding protein) (Purwana et al., 2014), a key transcription factor responsible for the maintenance of insulin gene transcription and β-cell survival in rodent and human islets (Shao et al., 2013). Among targets of CREB, the insulin receptor substrate-2 (IRS-2) may be a crucial gene in regulating β-cell mass and function. Studies in rodent islets or clonal β-cells suggest that glucose-induced up-regulation of IRS-2, in a Ca^{2+} and CREB-signaling dependent fashion is vital for β-cell plasticity in response to an increased demand for insulin (Purwana et al., 2014). The rhythmic control of insulin and glucagon release from pancreatic islets is crucial for maintaining blood glucose homeostasis and is regulated by GABA, which can be defective in T2D (Figure 1.12) (Rutter et al., 2019).

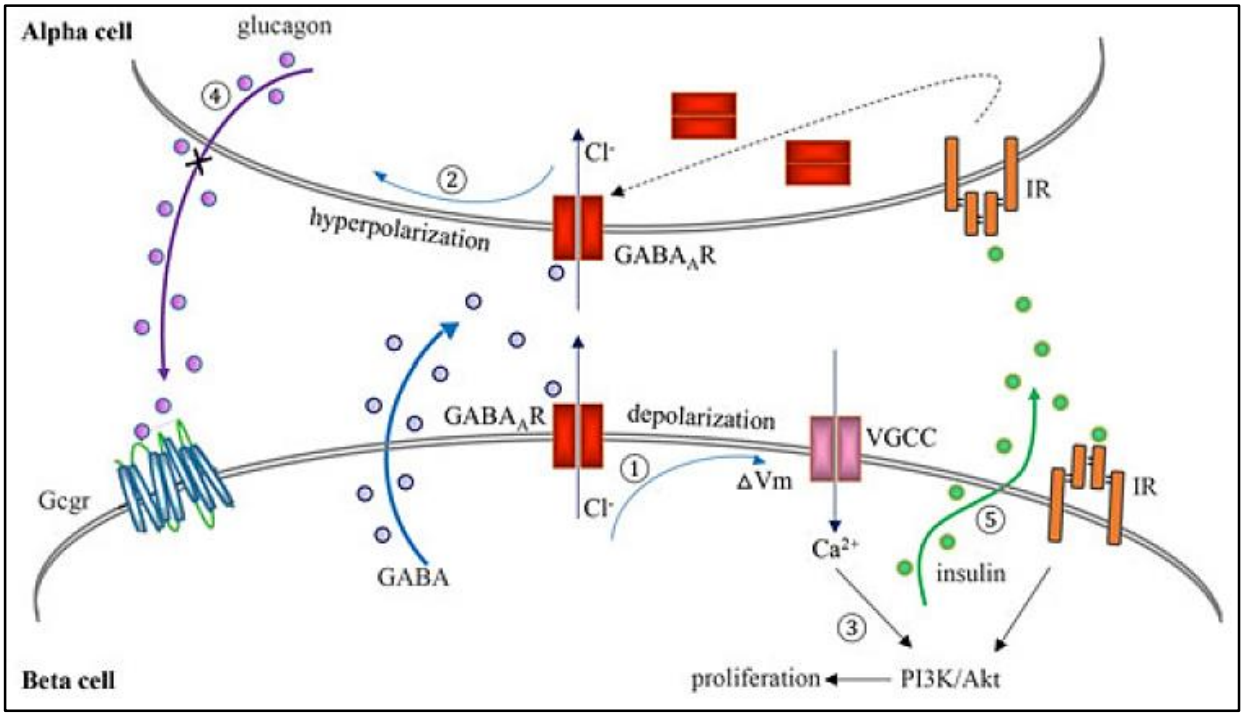

Fig. 1.12. Role of GABA in the regulation of pancreatic cell function. 1) GABA stimulates insulin release and β-cell proliferation *via* autocrine actions on β-cells. 2) GABA inhibits glucagon secretion by exerting paracrine effects on the α-cell. 3) Autocrine GABA promotes β-cell membrane depolarization, which activates the Ca^{2+}-dependent PI3K/Akt signaling pathway, which is crucial for cell proliferation and survival. 4) GABA induces paracrine hyperpolarization in α-cells, which suppresses glucagon activity. 5) GABA promotes autocrine insulin action. These physiologic mechanisms of GABA's autocrine and paracrine regulation may be disturbed in T1D and/or T2D receptor (Wang et al., 2019). [Abbreviations: Gcgr: glucagon]

Activating GABARs with GABA or specific agonists significantly inhibits β-cell apoptosis and protects β-cells in STZ-induced hyperglycemic mice following islet transplantation into NOD/SCID mice (Tian et al., 2013) and in isolated rat islets (Ligon et al., 2007). Similarly, GABA and GABAR agonists promote β-cell replication in hyperglycemic mice and human islets (Bansal et al., 2011; Wang et al., 2014; Wang et al., 2019). Tian et al. (2004) were the first to show the activity of GABA *in vivo* as an immunoprotective drug. They demonstrated the protective effects of GABA in non-obese diabetic (NOD) mice by suppressing activated T-cell responses against the islets. GABA is also reported to modulate the release of 47 cytokines from PBMCs of T1D patients and to suppress β-cell-reactive $CD8^+$ cytotoxic T lymphocytes (CTLs) in T1D models (Bhandage et al., 2018; Soltani et al., 2011; Wang et al., 2019). Another study demonstrated that long-term GABA administration in mice stimulated a significant boom in the number and size of islets, possibly due to the conversion of α-cells to β-like cells or through neogenesis of pancreatic ductal epithelial cells (Ben-Othman et al., 2017).

Furthermore, GABA has been reported to promote human β-cell replication and islet cell survival in humanized mouse model. The encouraging reports of GABA on T1D led to studies on T2D models as well. Combined GABA and sitagliptin (DPP-IV inhibitor) therapy have demonstrated β-cell regenerative effects in diabetic mouse models (Liu et al., 2017). All these studies indicate the therapeutic potential of GABA to stimulate the growth and function of insulin-producing β-cells and suggest that GABA can act as an immunosuppressive agent in diabetic therapy (Wang et al., 2019). Moreover, a few studies from the available literature showing the beneficial effects of GABA on metabolic profile/insulin sensitivity and the therapeutic potential of GABA in β-regeneration and apoptosis in pre-clinical and clinical trials are presented in Table 1.5.

Table 1.5. Therapeutic effects of GABA on metabolic profile, β-cell regeneration and apoptosis in a diabetes model.

Model	Strain/ Cell line	Dose of GABA and way of administration	Treatment Period	Effect	References
STZ-induced diabetes (40 mg/kg b.w. by i.p. for four consecutive days)	Male CD1 mice	NA	8-15 weeks	Activates PI3K/Akt-dependent growth and survival pathways. Restores the β-cell mass.	Soltani et al., 2011
STZ-induced diabetes (80 mg/kg b.w. i.p. two consecutive days)	Male C57BL/6 mice and STZ-induced diabetic NOD/SCID mice transplanted with human islets	2 or 6 mg/mL day in drinking water	14 days	Promotes β-cell replication and inhibits apoptosis in transplanted human islets.	Tian et al., 2013
NA	Female NOD mice	2, 6, or 20 mg/mL day in drinking water	20, 30, 45, and 50 weeks	Promotes β-cell replication.	Tian et al., 2014
STZ-induced diabetes (125 mg/kg b.w. i.p. two consecutive days)	Male NOD Mice transplanted with human islets	6 mg/mL/day in drinking water	5 weeks	Decreases blood glucose level and improves the glucose excursion rate. Promote β-cell proliferation and survival in transplanted human islets.	Purwana et al., 2014
STZ-induced diabetes (115 mg/kg b.w. by i.p.)	Male Wistar rats/ human islets	250 mg/kg b.w. by i.p	2 months	Promotes β-cell transdifferentiation in mice and α-to-β-like cell conversion in transplanted human islets.	Ben-Othman et al., 2017
STZ-induced diabetes	Male C57BL/6J mice	6 mg/mL/day in drinking	6 weeks	Decreases blood glucose level and improves the	Liu et al., 2017

(40 mg/kg b.w. by i.p. four consecutive days)		water		glucose excursion rate. Increases β-cell proliferation and reduces apoptosis.	
STZ-induced diabetes (125 mg/kg b.w. i.p. two consecutive days)	NOD/scid mice transplanted with human islets	6 mg/mL day in drinking water	10 weeks	Improves blood glucose levels and human plasma insulin. Increases transplanted human β-cell counts and protects against apoptosis.	Liu et al., 2021
10 weeks of HFD (60% kcal from fat)-induced obesity	Male CD1 mice	6 mg/mL day in drinking water	10 weeks	Promotes β-cell proliferation. Increased pancreatic β-cell mass led to a modest enhancement in insulin secretion and glucose tolerance.	Untereiner et al., 2019
HFD+STZ-induced T2D [20 weeks of HFD + STZ (40 mg/kg b.w. i.p. three consecutive doses)]	Male C57BL/6J mice	2.5 mg/kg b.w. by i.p	5 weeks	Induces β-cell regeneration and reduces apoptosis. Improves glucose tolerance, and insulin sensitivity	Rathwa et al., 2022
NA	PDESCs from the adult Sprague Dawley	50μM GABA in culture medium	28 days	Promotes differentiation of PDESCs into islet-like cell clusters containing β-like cells.	Cun et al., 2021
NA	Human islet	100μM GABA in culture medium	7 days	Increases the survival of human pancreatic islets by reducing apoptosis	Prud'homme et al., 2013
NA	INS-1 cells and human islets cells	100μM GABA in culture medium	48 hr.	Increases SIRT1 and NAD^+ levels, which in turn protects INS-1 and human islets cells from apoptosis	Prud'homme et al., 2014
20 weeks of HFD (60% kcal from fat)-induced T2D model	Male C57BL/6 mice	2 mg/mL/day in drinking water	12 weeks	Decreases FBG level. Improves glucose tolerance and insulin sensitivity. Inhibits body weight gain.	Tian et al., 2011b
20 weeks of HFD (45% kcal from fat)-induced T2D model	Male C57BL/6 Mice	0.6, 1.2, and 2 mg/mL day in drinking water	20 weeks	Improves blood glucose level and body weight gain. Alleviates HFD-induced plasma-free amino acids disorders	Xie et al., 2015
HFD+STZ-induced Diabetes [4 weeks of HFD (58% kcal from fat) +	Male Wistar rats	1.5 g/kg b.w. by i.p	12 weeks	Improves blood glucose level, insulin level, IPGTT, ITT, body weight, and body fat. Increases *GLUT4* gene & protein expression and	Sohrabipour et al., 2018

STZ (35 mg/kg b.w. i.p.)]				decreases the gluconeogenesis pathway and *Glucagon receptor* gene expression.	
HFD+STZ-induced T2D [3 months of HFD + STZ (35 mg/kg b.w. i.p.)]	Male and Female Wistar rats and offspring	1.5 g/kg b.w. by i.p	6 months and 4 months of treatment for offspring	Improves ITT, increases liver glycogen levels, decreases lipid profile and blood glucose level, HbA1c, and liver insulin resistance. Improved expression of *FOXO1*, *IRS2*, *Akt2*, and *PEPCK* genes.	Hosseini Dastgerdi et al., 2021
HFD+STZ-induced T2D [12 weeks of HFD (58% kcal from fat) + STZ (35 mg/kg b.w. i.p.)]	Male and Female Wistar rats and offspring	1.5 g/kg b.w. by i.p	6 months	Decreases blood glucose and HbA1c levels. Increases glucose infusion rate and IPGTT. Improves expression of *Akt*, *IRS1*, and *GLUT4* genes.	Rezazadeh et al., 2021

[Abbreviations: STZ: Streptozotocin; HFD: High-fat diet; SIRT1: Sirtuin 1; NOD/SCID: Nonobese diabetic/severe combined immunodeficiency; PI3K: Phosphatidylinositol-3-kinase; Akt: serine/threonine kinase; NAD+: Nicotinamide adenine dinucleotide, PDESCs: Pancreatic ductal stem cells; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; STZ: Streptozotocin; HbA1c: Haemoglobin A1c; PEPCK: Phosphoenolpyruvate carboxykinase; G6Pase: Glucose-6-phosphatase; HFD: High-fat diet; IPGTT: Intraperitoneal Glucose tolerance test; ITT: Insulin tolerance test; GLUT4: Glucose transporter type 4; IRS1/2: Insulin receptor substrate 1/2; Akt2: Serine/threonine kinases; FOXO1: Forkhead box protein O1.]

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