CHAPTER 1
INTRODUCTION

1. Introduction: Lifestyle Disorders

Like many other developing countries, India has witnessed a rapid epidemiological transition in the last two decades. Along with this, there has been a dramatic improvement of the Indian economy in terms of per-capita income. These dramatic changes have had a significant impact on the urbanisation and lifestyle of the Indians. As a result, many disorders, including diabetes mellitus (DM), cancer, reproductive diseases and metabolic syndrome have become the leading public health problems and amenable to change through early diagnosis at the individual level and surveillance population level [Kearney, 2010; Popkin *et al.*, 2012].

Diabetes Mellitus, a metabolic disorder, is characterised by persistent hyperglycemia with an increasing number of individuals diagnosed with diabetes. It is mainly classified into type 1 diabetes (T1D) and type 2 diabetes (T2D). Another rare form of diabetes, i.e., maturity-onset diabetes of the young, is directly inherited [Pramanik *et al.,* 2018; Rathwa *et al.*, 2020]. T1D constitutes <10% of total cases of diabetes worldwide, essentially initiated by autoimmune-mediated destruction of pancreatic β-cells, which often develops in childhood. On the contrary, T2D accounts for >90% of cases and is marked by insulin resistance in peripheral tissues due to impaired insulin signalling. Both forms of diabetes are associated with secondary complications that affect multiple organs [Pramanik *et al.,* 2018]. There are several factors associated with T2D, viz genetic predisposition [Dwivedi *et al.,* 2011; Patel *et al.,* 2016; Rathwa *et al.*, 2019], endoplasmic reticulum (ER) stress [Gregor *et al.,* 2011], obesity [Pramanik *et al.,* 2018, Gregor *et al.,* 2011] etc.

To date, extensive and innovative research has been carried out with a perspective to understand their molecular mechanisms and possible therapies. The world has witnessed various diabetes management approaches; however, a cure has not seen the light yet. The existing treatments only help alleviate hyperglycemia and other symptomatic characteristics. Gaining insights into the possible modes of β-cell preservation is crucial at this juncture. Hence an understanding of the signalling mechanisms of β-cell development and regeneration can open new treatment avenues.

1.1 The pancreas and insulin

The pancreas stems as an out pocketing of the primitive gut endoderm [Bonner-Weir *et al.*, 2012]. The adult mammalian pancreas is a varied organ formed of exocrine and endocrine cells; exocrine cells make up 95% of the pancreatic mass. In the histological sections of the pancreas, Islets of Langerhans are seen as relatively pale-staining cells embedded in darker-

staining exocrine tissue [Bastidas-Ponce *et al.,* 20017]. The islets are comprised of four endocrine cell types: β-cells which secrete insulin, α-cells which secrete glucagon, δ-cells which secrete somatostatin, and PP cells which secrete pancreatic polypeptide. β-cells form the bulk of cells (60%) in an islet forming a central core and surrounded by α-, δ- and PP cells. α-cells comprise 30% of the islet mass while δ and PP cells make up 10% [Da Silva Xavier, 2018]. Insulin has 51 amino acids with a molecular weight of 5802 Da. It is a dipeptide, containing A and B chains linked by disulphide bridges.

1.1.1 Mechanism of Insulin secretion

Amplified levels of glucose induce the "first phase" of glucose-mediated insulin secretion by release of insulin from the secretory granules in β-cells. Glucose entry into β cells is sensed by glucokinase that phosphorylates glucose to glucose-6-phosphate (G6P) to generate ATP [Koria *et al.,* 2013]. Closure of K^+-ATP-dependent channels results in membrane depolarization and stimulation of voltage dependent calcium channels causing K^+ ion outflow and elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). This is produced by an influx of extracellular Ca^{2+} via voltage-dependent Ca^{2+} channels, whose activity, in turn, is regulated by the β-cell membrane potential leading to a rise in intracellular calcium concentration and cause insulin secretion [Pramanik *et al.,* 2018; Rathwa *et al.,* 2020; Fu *et al.,* 2013].

1.1.2 Insulin signalling pathway

The cycle of feeding and fasting in healthy individuals do not modulate the plasma glucose levels which remain in a narrow range between 4 - 7 mM (70 to 120 mg/dL). It is stabilized by glucose absorption from the intestine, glucose production by the liver and glucose uptake from the plasma after meals. In peripheral tissues, glucose metabolism is controlled by insulin, whereas having no apparent role in stimulating glucose metabolism in brain, kidney, and erythrocytes. Insulin is also responsible for multiple actions: i) inhibits both basal and glucagon stimulated hepatic glucose production; ii) anabolic role of stimulating the storage of molecules in peripheral tissues by glycogen, lipogenesis and protein synthesis; iii) inhibiting lipolysis, glycogenolysis and protein breakdown; iv) stimulating cell growth and differentiation. Insulin mediates its actions through insulin receptor (IR) that activates a complex intracellular signalling network through insulin receptor substrate (IRS) proteins and the canonical Phosphoinositide 3-kinases (PI3K) and ERK cascades [Pramanik *et al.,* 2018; Rathwa *et al.,* 2020; Da silva Xavier, 2018; Fu *et al.,* 2013]. The IR consists of a heterotetramer of 2 α and 2 β glycoprotein subunits linked by disulphide bonds and is

located on the cell membrane. This receptor belongs to a subfamily of receptor tyrosine kinases that also includes the insulin-like growth factor-1 (IGF-1) receptor. The insulin receptor is present throughout the body, including in tissues classically considered as "responsive" and "nonresponsive" to insulin. Functionally, insulin binds to the α-subunit, initiates the activation and transphosphorylation of the β-subunits, leading to a conformational change and increase in kinase domain activity. These kinases phosphorylate tyrosine residues of adapter proteins called insulin receptor substrates (IRS) characterized by the presence of both pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains. The phosphorylated tyrosines in all of these substrates form specific sequence motifs and serve as "docking sites" for intracellular molecules that contain SH2 (Src-homology 2) domains. Thus, the insulin-receptor substrates act as main intermediates in insulin-signal transduction. The SH2 proteins that bind to phosphorylated IRS proteins fall into two major classes - the regulatory subunit of PI3-kinase or the molecule Grb2, which links with SOS to activate the Ras-mitogen-activated protein (MAP) kinase pathway. The other chief class of proteins that bind to IRS proteins are enzymes, such as the phosphotyrosine phosphatase SHP2 and cytoplasmic tyrosine kinases, such as Fyn. p85 or p55 regulatory subunit of PI3K (an adapter which has 8 isoforms) bind to IRS1/2 and activate PI3K pathway, resulting in activation of the p110 catalytic subunit (which has three isoforms) and generation of phosphatidylinositol-3,4,5-triphosphate (PIP3). This leads to activation of the three isoforms of AKT/PKB by PDK (phosphoinositide-dependent protein kinase) 1 and 2. PDKs bind to PIP3 in the cell membrane and get activated. There are four downstream substrates of AKT/PKB: i) mTOR, mammalian target of rapamycin, involved in the controlling of protein synthesis; ii) GSK3 (glycogen synthase kinase 3), involved in the modulating glycogen synthesis; iii) FoxO (forkhead box-containing protein, O subfamily) transcription factors, involved in the regulation of gluconeogenic and adipogenic genes and iv) AS160 (AKT substrate of 160kDa), involved in glucose transport. AS160 is a GTPase-activating protein which on phosphorylation activates small G proteins called RAB that are involved in membrane trafficking via blocking the exchange of GTP for GDP. Atypical Protein kinases C (PKCs) isoforms are also involved in downstream of PDK1 that induces GLUT4 translocation to the membrane for glucose uptake [Gregor, 2011; Bonner-Weir *et al.*, 2012; Bastidas-Ponce *et al.*, 217; Fu *et al.*, 2013].

1.2 Epidemiology of Diabetes Mellitus

It is estimated that worldwide by 2045, 700 million adults will have diabetes, a 51% increase in the cases [International Diabetes Atlas, 2019] and T2D accounts for >80% of the diagnosed cases. These numbers are shooting up with increasing urbanisation and sedentary lifestyles (Figure 1.1). It is estimated that by 2025, 69.9 million Indians will be diagnosed with diabetes, and 8.5% accounts for undiagnosed cases (Figure 1.1) [International Diabetes Atlas, 2019]. A few reports indicate Gujarat state with the second most T2D patients accounts for 10.7% of newly diagnosed cases [Koria *et al.*, 2013; Anjana *et al.*, 2017].

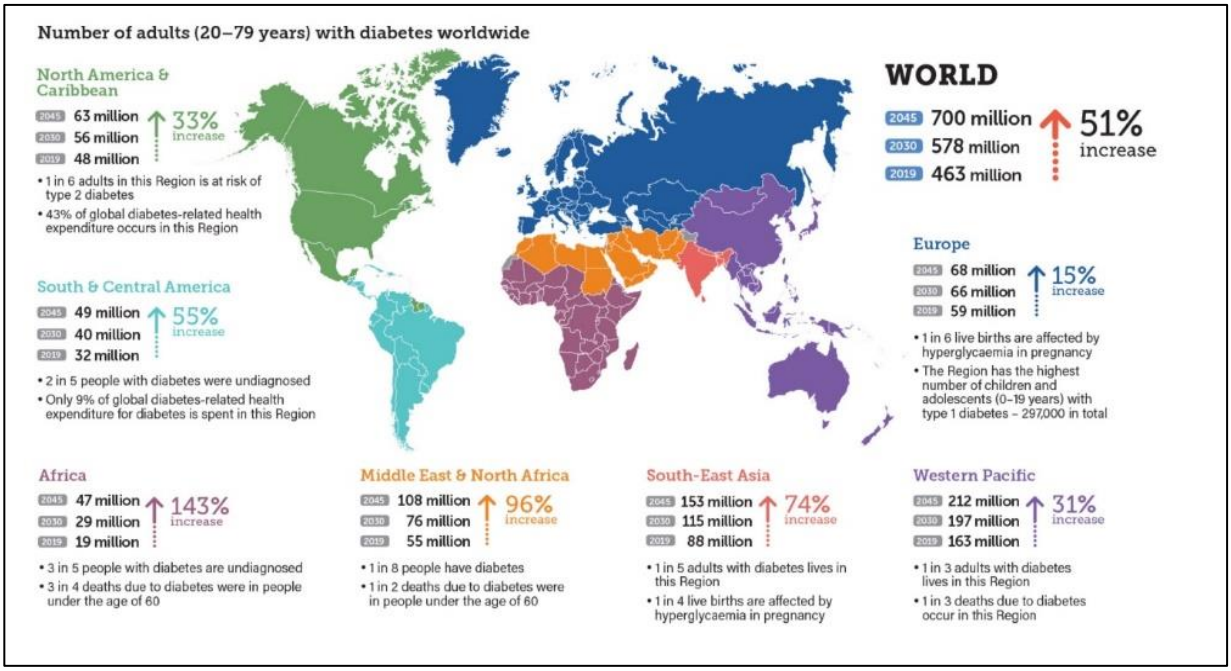

Figure 1.1: Epidemiology of Diabetes Mellitus: Worldwide diagnosed diabetic cases are more than 700 million, with a 51% increase in cases. Looking closely at South-East Asia, it forms close to 153 million cases, at an alarming rate of 74%. There is 1 in 5 adults affected with diabetes in this targeted region and 1 in 4 affected by gestational diabetes.

1.2.1 Symptoms, classification, diagnosis and pathogenesis of Diabetes Mellitus

Many diabetes cases can be categorised into T1D and T2D. Gestational diabetes mellitus (GDM) indicates impaired glucose tolerance (IGT), first diagnosed during pregnancy. "Prediabetes" is a term referred to impaired fasting glucose (IFG), IGT or glycated haemoglobin (A1C) that predisposes an individual to a high risk of developing diabetes and its complications (Table 1.1) [Goldenberg *et al.*, 2013; Tomita *et al.*, 2017]. The typical symptoms of DM are frequent urination, thirst, fatigue, hunger, blurred vision, cuts/bruises that are slow to heal, weight loss (T1D), tingling, pain, or numbness in the hands/feet (T2D). However, some people diagnosed with T2D may not notice any of these symptoms.

Table 1.1: Characteristics of prediabetes*

Impaired Fasting Glucose	Fasting plasma glucose: 100 mg/dL - 125 mg/dL (5.6 mmol/L - 6.9 mmol/L)
Impaired Glucose Tolerance	2 hours plasma glucose in the 75 g oral glucose tolerance test: 140 mg/dL - 199 mg/dL (7.8 mmol/L - 11.0 mmol/L)
A1C	5.7 – 6.4 %

*For all three tests, the risk is continuous, extending below the lower limit of the range and becoming disproportionately more significant at higher ends of the spectrum [Lieberman *et al.*, 2003].

1.3.1 Type 1 Diabetes

T1D occurs due to the autoimmune destruction of the pancreatic β-cells and consists of <10% of the diagnosed cases. T1D is also labeled as insulin-dependent diabetes mellitus (IDDM) which may influence a person of any age but mostly detected in children and adolescents. Genetic susceptibility is crucial in the development of T1D. The lifetime risk for an individual in the population is often cited as 0.4%. The risk is >1% if the mother has diabetes and rise to >3% if the father has T1D. Earlier reports as show that having specific combinations of genes is not sufficient to cause T1D. Environmental triggers consequently modulate the onset of T1D in genetically susceptible people. Environmental reasons have been involved in the recent rapid increase in T1D frequency because the gene pool remains stagnant and do not account for the rapid rate of increase of T1D [Pramanik *et al.*, 2018; Rathwa *et al.*, 2020; Da Silva Xavier, 2018].

Two popular theories explain the mechanism behind T1D. In the autoimmune-mediated mechanism, macrophages and dendritic cells are the first cell types to infiltrate the pancreatic islets. These macrophages and dendritic cells present major histocompatibility complex (MHC) and β-cell peptides to naive CD4+ T cells that circulate in the blood and lymphoid organs. Concurrently, activated TH1 CD4+ T cells produce IL-2 that activates β-cell antigen-specific CD8+ T cells which further differentiate into cytotoxic T cells and get incorporated into the pancreatic islets inducing the destruction of β-cells. Furthermore, CD8+ T cells and activated macrophages release granzymes, perforin, cytokines and reactive oxygen species (ROS). Thus, by and large, all act synergistically in destroying β-cells leading to autoimmune diabetes [Tomita *et al.*, 2017]. The role of insulin resistance in T2D is well known, but recent reports suggest insulin resistance could affect at the level of skeletal muscle and liver in T1D also in two ways.

I) Skeletal muscle insulin resistance is due to decreased glucose transport into myocytes impairing insulin sensitivity. Serine phosphorylation of IRS-1 leads to insulin resistance in obese T1D individuals due to the ectopic fat. The increased levels of intramyocellular lipids (IMCLs) and plasma free fatty acids (FFAs) activate serine kinase i.e., IκB kinase-β, which preferentially phosphorylates serine on IRS-1 causing decreased glucose transport [Pramanik *et al.*, 2018; Tomita *et al.*, 2017].

II) The liver adjusts glucose homeostasis by gluconeogenesis, glycogenolysis (fasting state) and glycogenesis (fed state). Insulin suppresses gluconeogenesis via inhibition of PEPCK, promoting glycogen synthesis via stimulus of GSK-3, and inhibition of G6Pase [Czech, 2017].

1.3.2 Type 2 Diabetes

T2D is characterised by insulin resistance which ranges from relative insulin deficiency to a predominantly secretory defect [Pramanik *et al.*, 2018]. Since patients are not completely dependent on exogenous insulin, T2D is termed as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. This form of diabetes is not diagnosed for many years as the hyperglycaemia is often weak to create effective symptoms of diabetes. Nevertheless, such patients are at greater chance of developing macrovascular and microvascular complications [Pramanik *et al.*, 2018; Rathwa *et al.*, 2020]. The clinical features of T1D and T2D patients (Table 1.2) and their diagnosis (Table 1.3) are mentioned below.

Table 1.2: Clinical characteristics of T1D and T2D patients [Rotella *et al.*, 2013].

Features	**Type 1 Diabetes**	**Type 2 Diabetes**
Age of onset	Usually <20 year	Generally over 30 years
Body Mass	Low to normal	Obese
Plasma Insulin	Low or absent	Normal to high initially
Plasma Glucagon	High, can be suppressed	High, resistant to suppression
Plasma Glucose	Increased	Increased
Insulin sensitivity	Normal	Reduced
Therapies	Insulin	Oral medications, i.e., metformin, thiazolidinediones, sulfonylureas

Table 1.3: Criteria for the diagnosis of diabetes [American Diabetes Association, 2014]

Diagnostic Test	**Normal**	**Diabetes Mellitus**	
Haemoglobin	<5.7%	≥6.5%	The test should be done in

A1C			a lab using a government certified method and standardised to the Diabetes Control and Complications Trial assay.*
Fasting plasma glucose	<100 mg/dL (5.5 mmol/L)	≥126 mg/dL (7.0 mmol/L)	Fasting is defined as no caloric intake for at least 8 hours
OGTT	<140 mg/dL (7.8 mmol/L)	2 hours plasma glucose ≥200 mg/dL (11.1 mmol/L)	The test should be done as defined by the World Health Organization, using a glucose load having the equivalent of 75g anhydrous glucose dissolved in water.*
Random plasma glucose	<130 mg/dL (7.2 mmol/L)	≥200 mg/dL (11.1 mmol/L).	In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be definite by repeat testing.

1.3.2.1 Mechanism involved in Type 2 Diabetes

There are several hypotheses put forth to explain the molecular mechanism of T2D. In T2D patients, various factors such as inflammation, obesity, ER stress and mitochondrial dysfunction trigger insulin resistance. The impaired insulin signalling pathway leads to severe complications [Czech, 2017]. Over nutrition and sedentary lifestyle contribute to hyperlipidemia and insulin resistance causing hyperglycemia. This condition modifies cellular metabolism and intracellular signalling that deleteriously impact cells. In the skeletal muscle, this damage can be reviewed into three actions: i. modification in insulin signalling, ii. amplified substrate accessibility, iii. inflexibility in metabolic changes. The downstream cellular events including i. gene expression modifications, ii. hyperglycemia and dyslipidemia, iii. activation of oxidative stress and inflammatory response, iv. ectopic lipid accumulation, which is favoured by obesity delaying the metabolic deregulation [Ormazabal *et al.*, 2018].

Chronic exposure to aberrant hyperglycemia has harmful effects on cell survival, insulin secretion and sensitivity (Figure 1.2). It is mediated through glucotoxicity leading to persistent β-cell deterioration due to caspase-mediated apoptosis as one of the crucial factors [Samuel and Shulman, 2012].

I) In skeletal muscle, chronic hyperglycemia leads to a long-term increase in cytosolic Ca^{2+} and mitochondrial dysfunction resulting into apoptosis. It decreases the number of mitochondria and alters their morphology, impaired glucose-stimulated insulin secretion through impaired oxidative phosphorylation, decreased mitochondrial Ca^{2+}, and reduced ATP generation. These alterations lead to the activation of apoptotic pathways [Samuel and Shulman, 2012; Szpigel *et al.*, 2018].

II) Hyperglycemia increases the metabolic flux into the mitochondria and induces excessive ROS generation, leading to oxidative stress. Mitochondrial oxidative phosphorylation, glucose auto-oxidation, non-enzymatic glycation, PKC activation, and various metabolic pathways produce excessive ROS. It also damages the β-cells by inducing defective insulin biosynthesis and secretion, and ultimately apoptosis. Further, disruption of mitochondrial membrane integrity and mitochondrial DNA mutations promote apoptosis [Pramanik *et al.*, 2018; Rathwa *et al.*, 2020, Czech 2017].

III) Increasing demand for insulin stresses β-cells, and their ER to produce more proinsulin. ER stress results in an accumulation of unfolded proteins and activates the unfolded protein response. It may cause β-cell apoptosis (mediated by stress kinases and transcription factors) [Samuel and Shulman, 2012].

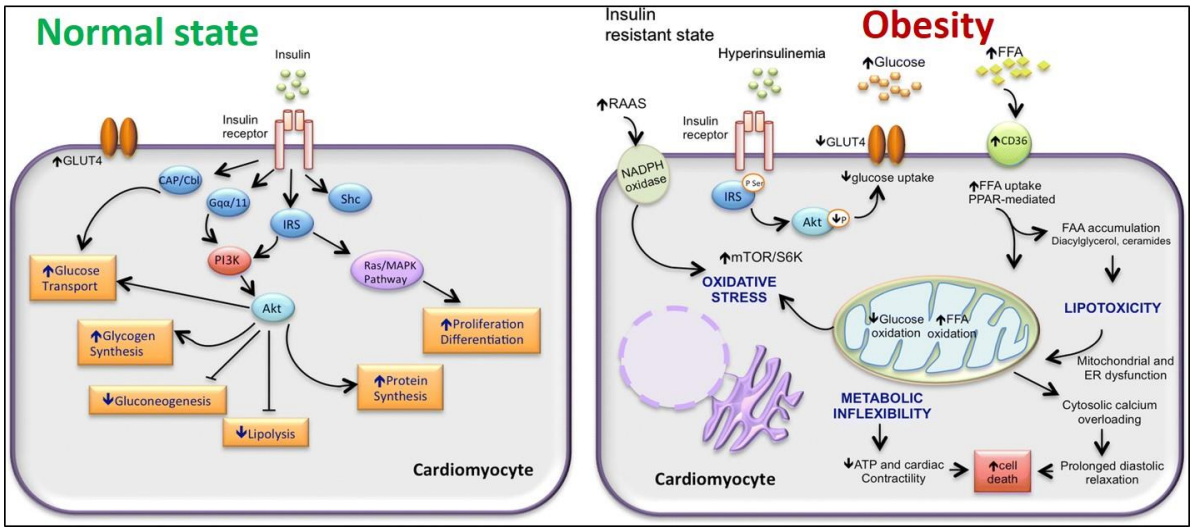

Figure 1.2. Mechanisms implicated in the insulin resistance. In healthy state, the insulin signalling controls the glucose and lipid metabolism. Insulin resistance produces a metabolic derangement that results in high lipid oxidation, and low glucose oxidation. The activation can induce mitochondrial dysfunction, ER stress and oxidative stress, resulting in decreased mitochondrial Ca^{2+}, low ATP production, leading to apoptotic pathways. Adapted from Ormazabal *et al.*, 2018 [Ormazabal *et al.*, 2018].

1.3.2.2 Role of adipose tissue and liver in Type 2 Diabetes

Adipose tissue (AT) serves as energy store house which is no longer considered inert. Moreover, it has emerged as a dynamic tissue involved in regulating physiological and pathogenic processes, including immunity and inflammation. Inflammation in AT is one of the mechanisms to induce insulin resistance, which is facilitated by the stimulation of cellular-stress which produce inflammatory signalling paths. Macrophages in AT actively modulate these processes. Further, crosstalk between lymphocytes and adipocytes can lead to immune regulation. Adipokines are hormones/cytokines secreted by adipose tissue with a role in the "adipo-insular" axis. Some adipocytokines (such as TNF-α, leptin, resistin) act as pro-inflammatory and contribute to β-cell failure; others are anti-inflammatory adipocytokines (vaspin, omentin-1, adiponectin) and exert protective effects on β-cell function and survival [Rathwa *et al.*, 2019, Patel *et al.*, 2019; Palit *et al.*, 2020; Rathwa *et al.*, 2019; Rathwa *et al.*, 2020]. Altered adipokine levels have been observed and implicated in obesity-induced T2D pathophysiology (Figure 1.3).

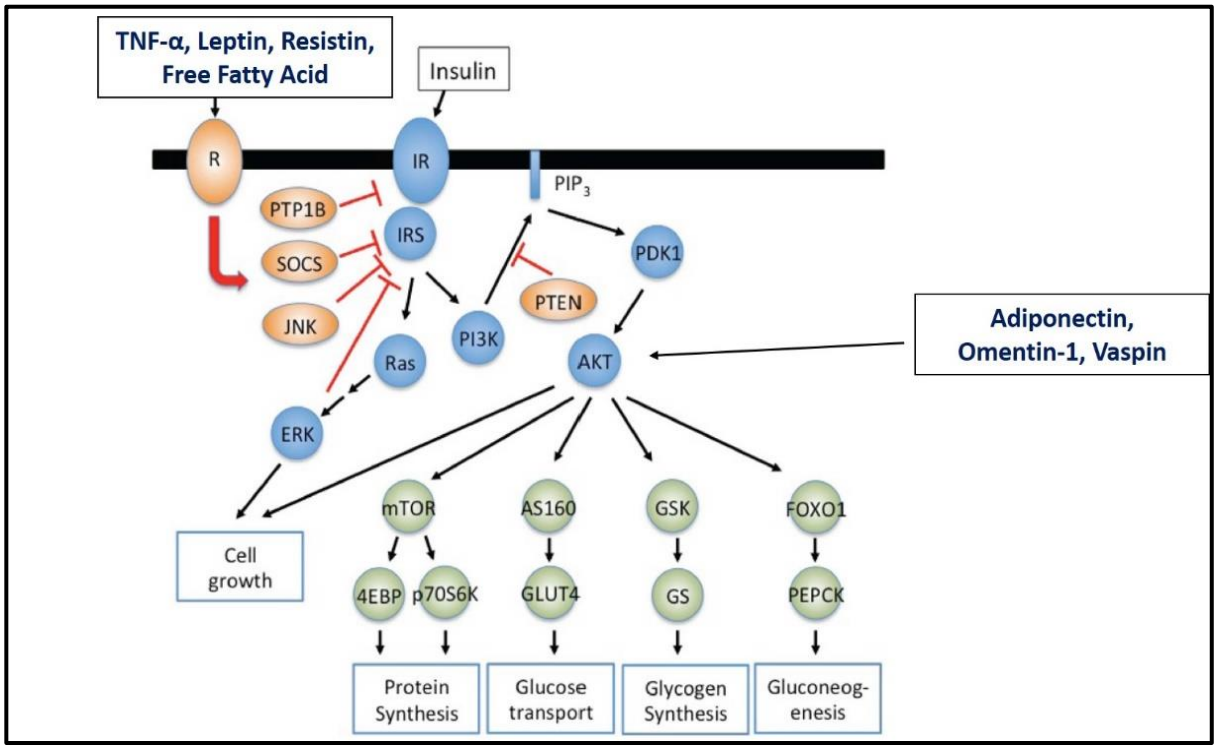

Figure 1.3. Roles of adipokines in insulin signalling: IRS1/2 phosphorylated on specific tyrosine residues to activate the PI3K-Akt/PKB pathway and Ras-mitogen-activated protein kinase (MAPK) pathway. PI3K-Akt signalling pathway regulates metabolic processes such as glucose uptake (muscle and adipocytes), glycogen synthesis (muscle and liver), protein synthesis (muscle and liver), and gluconeogenesis (liver). Pro-inflammatory adipokines TNF-α, leptin, resistin, and saturated FFAs activate inhibitory molecules such as SOCS

(Suppressor of cytokine signaling) and c-Jun N-terminal kinase (JNK) to suppress insulin signalling resulting in insulin resistance. Anti-inflammatory cytokines enhance insulin sensitivity *via* direct/indirect action of the PI3K-Akt pathway. Adapted from Pessin *et al.*, 2013 [Pessin and Kwon, 2013].

Hepatocytes initially metabolize carbohydrates absorbed from the intestine. In contrast, dietary fatty acids form triacylglycerols in the enterocytes, and reach the lymph stream assembled as chylomicrons, then come into the bloodstream and finally enter the liver as remnant chylomicrons. VLDL, a very low-density lipoprotein, is produced in the liver, which depends on the availability of substrates and is tightly regulated by insulin. Hepatic VLDL production is induced in the fasting state, and elevated levels are observed in the blood. Moreover, lipids from different sources, such as circulating FFA, endocytosis of triglyceride-rich lipoproteins, and *de novo* lipogenesis, allow for posttranslational stabilization of apoB and enhance the assembly and secretion of VLDL particles. This leads to VLDL and FFA production, which carries cholesterol between the liver and the adipose tissue. In the healthy state, insulin, through PI3K activation, promotes degradation of apoB, but under insulin resistance, this degradation is impaired. Thus, the deadly combination of i) excessive FFAs, ii) limited apoB degradation, and iii) stabilization of apoB; result in increased VLDL synthesis that leads to hypertriglyceridemia. Prolonged exposure of amplified FFA levels elicits toxic FA metabolites leading to "lipotoxicity" (Figure 1.4) and β-cell death [Ormazabal *et al.*, 2018] as mentioned below:

I) FA-induced protein kinase B inhibition in β-cell leads to the downregulation of the anti-apoptotic factor Bcl2, and excessive *de novo* ceramide synthesis is also involved.

II) Ceramide activates NF-κB, which upregulates inducible nitric oxide synthase (iNOS). It enhances nitric oxide and peroxynitrite formation and inhibits the mitochondrial respiratory chain complexes to promote ROS mediated apoptosis [Szpigel *et al.*, 2018].

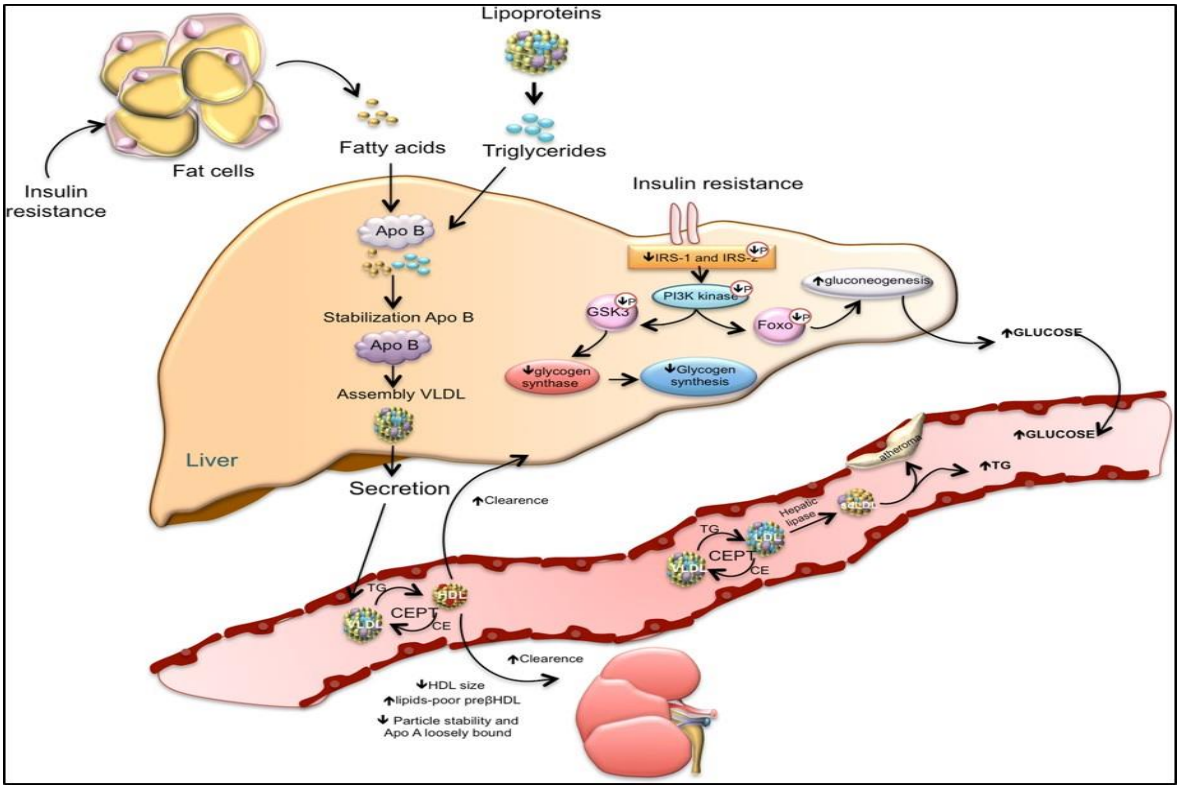

Figure 1.4. A simplified model of insulin resistance. The loss of suppressive effects of insulin on lipolysis pathway in AT increases FFAs. Increased FFAs flux to the liver stimulates the assembly and secretion of VLDL causing in hypertriglyceridemia. Triglycerides in VLDL are transported to both LDL and HDL by cholesteryl ester transfer protein (CETP). Triglyceride-enriched HDL is rapidly cleared from the circulation by kidney. However, in insulin resistance condition there is decreased hepatic glycogen synthesis, owing to decreased activation of glycogen synthase, increased hepatic gluconeogenesis, and glucose delivery by the liver. Adapted from Ormazabal *et al.,* 2018 [Ormazabal *et al.,* 2018].

1.4 Role of genetic factors in Type 2 Diabetes

Most human diseases result from an interaction between genetic variants and environmental factors. Establishing the contribution of genetic variants, is the first step in genetic studies to evaluate complex conditions. In general terms, the search for genetic components begins through observational studies, such as analysis of pools of familial cases, comparative studies of the rate of disease occurrence among monozygotic and dizygotic twins and complex segregation analysis (CSA). To advance, it is essential to perform different tasks involving molecular genetic markers, as it is done in association and linkage analysis. Linkage studies for T2D have provided promising and replicative findings. These robust replications suggested the plausibility of localising single or multiple susceptibility genes on

these chromosomal regions [Pramanik *et al.,* 2018; Patel *et al.,* 2004; Nagaev *et al.,* 2001]. Various lines of evidence support the view that genetic components play an important role in the pathogenesis of T2D:

(i) The prevalence of T2D varies widely among populations. Part of the observed ethnic variability is attributed to non-genetic environmental factors. However, the observation that the disease prevalence varies substantially among ethnic groups but still share a similar environment supports the idea that genetic factors contribute to predisposition to the disease.

(ii) Familial aggregation of the disease is another source of evidence for genetic contribution to the disease, although, families also share common environmental traits. The odds ratio for offspring of a single affected parent is 3.5 compared to those with no parental diabetes history, and the odds ratio increases to 6.1 if both parents are affected.

(iii) The high concordance in monozygotic twins (>80%) and the 50% decline in dizygotic twins provide compelling evidence for genetic components in the aetiology of T2D.

(iv) Data from various studies are in support of a genetic basis that measures of both insulin sensitivity and insulin secretion [Pramanik *et al.,* 2018; Patel *et al.,* 2004; Nagaev *et al.,* 2001].

Recent population-based studies reveal a rising prevalence of diabetes in India's urban areas ranging between 12-16%. While environmental factors certainly play a significant role, this usually occurs on a background of genetic susceptibility. Mohan et al. first indicated that plasma insulin levels are higher in Asian Indians than Europeans [Anjana *et al.,* 2011]. Later it was revealed that Asian Indians are more insulin resistant. Though the body mass index (BMI), an indicator of obesity, is lower among Indians, for any given BMI, the waist to hip ratio was higher among Indians compared to other ethnic groups [Anjana *et al.,* 2011].

Further, at any BMI, Indians also had higher body fat; and even when matched for body fat, Indians had more significant insulin resistance than other ethnic groups. These studies suggest that Indians seem to be genetically more prone to diabetes and insulin resistance. This indicates that Indians have a propensity to diabetes, probably due to their genetic predisposition [Anjana *et al.,* 2017].

1.5 Role of Resistin in Type 2 Diabetes

Resistin gene, located on chromosome 19 of mouse/human, was initially recognised in 2001. Resistin (12.5 kDa) is an unusual hormone with 11 cysteine residues out of a total of 114 amino acids [Filková *et al.,* 2009; Steppan *et al.,* 2001]. In serum, resistin circulates predominantly as trimer and hexamer, with the trimer being the most bioactive form [Patel

et al., 2004]. It is expressed at low levels in human adipose tissue, whereas high levels are expressed in bone marrow, spleen, macrophages and mononuclear leukocytes [Nagaev *et al.*, 2001; Steppan *et al.*, 2001; Patel *et al.*, 2004]. Studies have suggested that mature human adipocytes lack resistin expression, while preadipocytes show resistin expression [Janke *et al.*, 2002; Fain *et al.*, 2003]. Infusion of resistin in Sprague-Dawley rats resulted in weakened hepatic insulin sensitivity and glucose metabolism [Rajala *et al.*, 2003]. Chronic high circulating resistin levels led to increased fasting plasma glucose, weakened glucose tolerance, and reduced hepatic insulin sensitivity [Rangwala *et al.*, 2004]. Resistin induces insulin signalling impairment by activating SOCS3 in adipocytes [Steppan *et al.*, 2005]. Several studies have shown a positive correlation between elevated resistin levels, and insulin resistance and obesity in humans. Resistin was identified as a pro-inflammatory adipokine mediating its action via TNF-α by activating the NF-κB pathway [Silswal *et al.*, 2005] and recruiting immune cells [Pramanik *et al.*, 2018]. A recent study unravelled that resistin binds to adenyl cyclase associated protein 1 receptor, increasing cAMP and PKA activity [Lee *et al.*, 2014]. Steppan *et al.* [Steppan *et al.* 2005] showed that blocking the action of resistin with neutralising antibody was found to improve whole-body insulin sensitivity in the diet-induced obese (DIO) mouse model, while antisense oligodeoxynucleotides for resistin mRNA biomarker contribute to insulin resistance in specific populations (Figure 1.5).

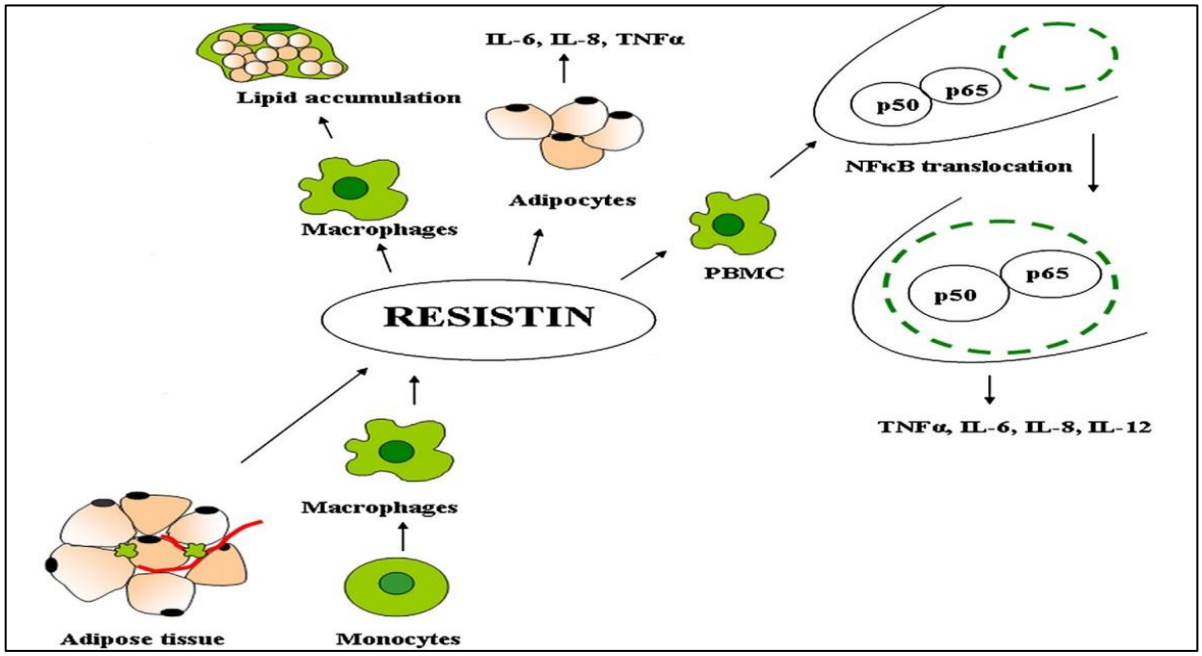

Figure 1.5. Resistin as a potential regulator of inflammation. A schematic representation of crucial pathophysiological signalling pathways mediated by resistin in immune and

resident tissue cells. Resistin activates the transcription factor NF-κB. It is upregulated during monocyte-macrophage differentiation and increases after TNF-α, IL-1β, IL-6, and LPS stimulation. It induces the expression of several pro-inflammatory cytokines and lipid accumulation. Abbreviations: IL, interleukin; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharide. Adapted from Filková *et al.*, 2009 [Filková *et al.*, 2009].

1.5.1 Genetic variants of *resistin*

Many research groups have examined genetic variants in resistin, and it is assessed that up to 70% of the variation in circulating resistin levels are due to genetic factors [Pramanik *et al.*, 2018; Gregor, 2011]. Interestingly, association between resistin and BMI or other adiposity markers have shown inconsistent results [Pramanik *et al.*, 2018; Gregor, 2011; Filková et al., 2009; Fain *et al.*, 2003]. Polymorphisms in resistin also have been associated with indexes of insulin resistance, but lack of replication and null associations have raised questions regarding their consistency. Moreover, most of the studies examining common variations in resistin and risk of T2D have been negative [Pramanik *et al.*, 2018; Gregor, 2011; Filková et al., 2009; Fain *et al.*, 2003]. These inconsistencies might be due to low power of the studies due to small sample size or inadequate coverage of the gene and its flanking sequences. The prominent genetic variants of resistin in various populations are depicted in the Table 1.4 [Rathwa *et al.*, 2019].

Table 1.4. Association of *resistin* genetic variants with various disorders.

Sr. No.	Genetic variant	Associated with	Population
1.	rs1862513	T2D	Japanese, Korean, Indian
2.	rs1477341, rs4804765 rs1423096, rs10401670	T2D	USA
3.	rs1862513	Metabolic, obesity syndrome, kidney dysfunction, myocardial infarction	Italian
4.	rs3745369	Obesity	Danish

1.6 Role of Omentin-1 in Type 2 Diabetes

Omentin-1 is a novel 40 kDa fat depot-specific adipokine, which has been identified from a cDNA library of visceral omental adipose tissue, located on 1q21.3 chromosome locus [Ohashi *et al.*, 2014]. It is also known as intelectin-1, intestinal lactoferrin factor, endothelial lectin or galactofuranose binding lectin. Omentin is expressed as two homologous proteins,

omentin-1 and -2, encoded by two separate genes located adjacent to one another on 1q22-q23 in humans. Omentin-1 is the key circulating isoform in human plasma. Omentin-2 shows 83% amino acid identity with omentin-1 [Pramanik *et al.*, 2018]. Omentin mediated glucose uptake occurs via the phosphorylation of Akt at physiological concentrations [Yang *et al.*, 2006]. Omentin gene expression in visceral adipose tissue and circulating omentin levels were reported to decrease in obese individuals, however signalling mechanism remains obscure. Nevertheless, the above studies indicate diabetic patients [Pramanik *et al.*, 2018] associated with IGT and T2D [Pramanik *et al.*, 2018; Yang *et al.*, 2006].

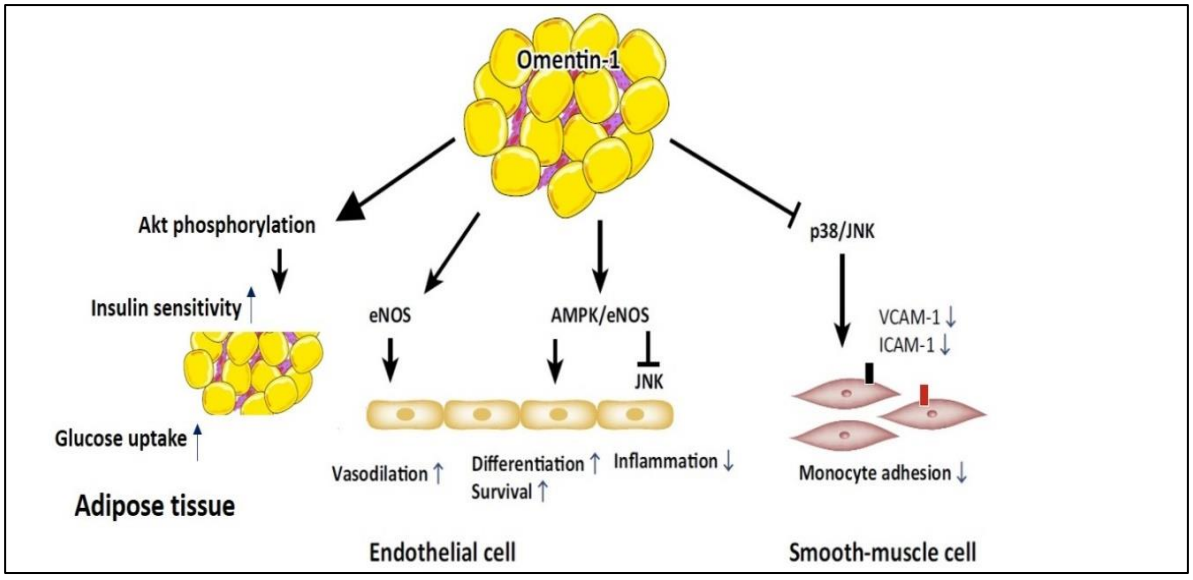

Figure 1.6. The protective function of omentin-1. Omentin-1 exerts anti-inflammatory and anti-atherogenic functions in AT, endothelial cells and smooth muscle cells. Omentin-1 enhances Akt phosphorylation to increase insulin sensitivity. It promotes vasodilation and endothelial cell differentiation and survival by activating the AMPK/eNOS pathway. Omentin-1 inhibits inflammation in endothelial cells by suppressing JNK activation through an AMPK/eNOS-dependent pathway. Omentin-1 also attenuates monocyte adhesion to smooth-muscle cells by reducing VCAM-1 and ICAM-1 by suppressing p38/JNK signalling. Adapted from Ohashi *et al.*, 2014 [Ohashi *et al.*, 2014].

1.6.1 Genetic variants of *omentin-1*:

There are a few studies on the genetic variants of *Omentin-1* wherein Val109Asp rs2274907 has been exclusively studied in Non-alcoholic Fatty Liver Disease (NAFLD) [Pramanik *et al.*, 2018], Coronary Artery Disease (CAD), psoriasis, high calorie-diet intake, breast cancer and rheumatoid arthritis. There is only one report on *Omentin-1* 3' UTR rs1333062 in the Indian population showing an association with diabetes [Pramanik *et al.*, 2018; Ormazabal *et al.*, 2018].

1.7 Role of Vaspin in Type 2 Diabetes:

Vaspin, a visceral adipose tissue (VAT) derived serine protease inhibitor, has an insulin-sensitising effect and belongs to the serpin superfamily (Serpina12) and it is located on chromosome 14. It was found in the VAT of Otsuka Long-Evans Tokushima Fatty rat (OLETF), central obesity and T2D mouse model [Hida *et al.*, 2005]. Vaspin acts as a circulating serpin, which aids as a ligand for a cell-surface receptor complex, GRP78/MTJ-1, and exerts anti-inflammatory action in ER induced stress [Nakatsuka *et al.*, 2012]. Nakatsuka et al. [Nakatsuka *et al.*, 2013] showed vaspin's ligand property in the endothelial cells for a cell-surface voltage-gated anion channel complex, thereby exerting anti-apoptotic, proliferative, and protective effects in T2D rat models. Furthermore, vaspin also protects endothelial cells by inhibiting NF-κB [Pramanik *et al.*, 2018; Heiker, 2014]. Elevated vaspin was associated with obesity in young Korean men with BMI, triglycerides, fasting insulin, and insulin resistance in pubertal obese children [Pramanik *et al.*, 2018]. However, the administration of recombinant vaspin in obese mice showed improved glucose tolerance and insulin sensitivity, suggesting the rise in vaspin levels to be a compensatory increase in response to obesity and insulin resistance. Interestingly, it was also higher in healthy females than healthy males demonstrating sexual dimorphism [Pramanik *et al.*, 2018; Rathwa *et al.*, 2020]. On the other hand, several studies have failed to show correlation between serum vaspin levels and BMI and insulin sensitivity [Pramanik *et al.*, 2018] or with T2D [Pramanik *et al.*, 2018]. Interestingly, vaspin influences insulin-induced glucose uptake *in vivo*, but not *in vitro*. Vaspin probably modulates insulin action only on its target proteases, which perhaps triggers altered insulin sensitivity. Therefore, the identification of vaspin's target protease is the major challenge for future studies related to vaspin. Unravelling the proteases might lead to novel antidiabetic therapy, which may improve insulin sensitivity in patients with T2D (Figure 1.7).

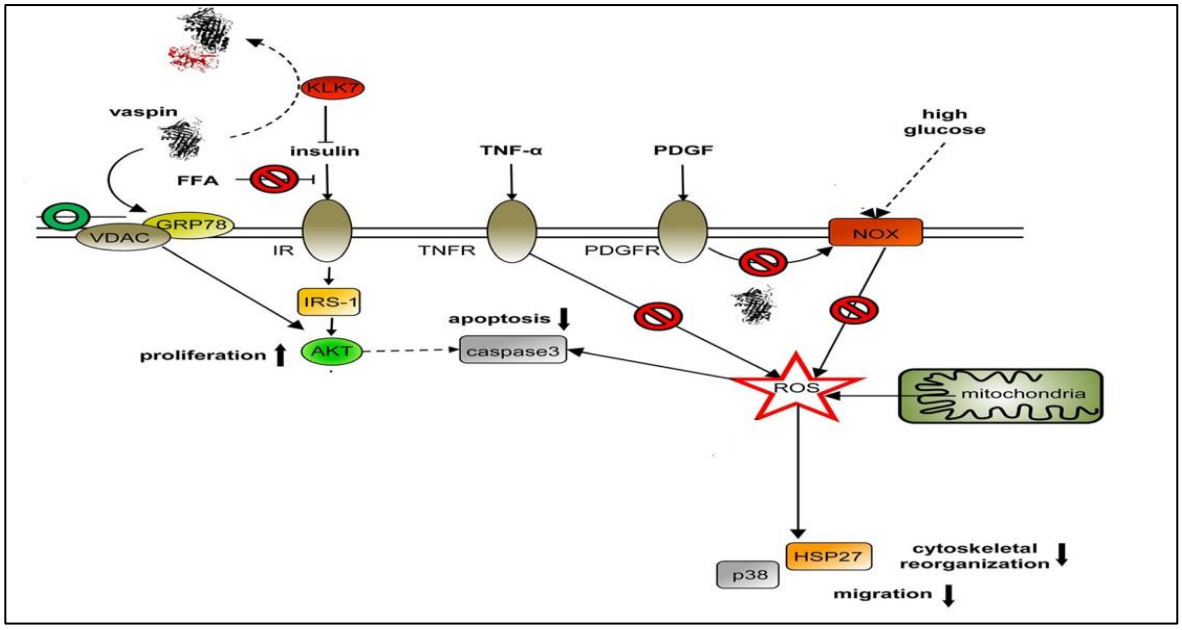

Figure 1.7. Anti-inflammatory and anti-apoptotic effects of vaspin. Vaspin modulates several molecular pathways and shows its beneficial effects in endothelial and vascular smooth muscle cells (pathways impaired are marked with a red symbol and induced effects with a green sign). Vaspin inhibits TNF-α, platelet-derived growth factor (PDGF), and high glucose-induced ROS formation and successively inhibits cell apoptosis (reduced caspase-3 activity), cytoskeletal reorganisation, and migration (reduced expression of activation of p38 and HSP27). Vaspin increases Akt signalling to promote proliferation. Adapted from Heiker *et al.*, 2014 [Heiker *et al.*, 2014].

1.7.1 Genetic variants of *vaspin*:

Several SNPs within and around the *vaspin* gene have been identified to influence disease and vaspin levels. While some SNPs do not alter the vaspin coding sequence and protein structure, activity, or half-life, the rare *vaspin* functional variant rs61757459 results in a truncated protein to an early stop codon and therefore affects circulating vaspin levels [Pramanik *et al.*, 2018; Rathwa *et al.*, 2020]. A significant association of *vaspin* SNP rs2236242 with T2D has been reported in 2,759 participants of the KORA F3 study. Kempf *et al.* found that the AA genotype is independent of obesity-associated increased diabetes risk and suggests *vaspin* as a candidate gene for impaired glucose metabolism. This particular genetic variant is studied with other disorders (Table 1.5) [Rathwa *et al.*, 2020].

Table 1.5. Association of *vaspin* rs2236242 A/T with various disorders.

Sr.No.	Associated with	Population
1.	T2D	German, Chinese

2.	CAD	Chinese
3.	Obesity	Egyptian
4.	Metabolic Syndrome	Egyptian, Iranian
5.	PCOS	Iranian
6.	ESRD	Iranian

1.8 β-cell Regeneration

The mammalian pancreas is a gland of the digestive tract, composed of about 99% of exocrine cells and 1% of endocrine cells. The adult pancreatic cell mass is determined during development phase by a small pool of endodermal PDX1+ progenitor cells distributed in two primordial (dorsal and ventral) [Rathwa *et al.,* 2020]. These progenitor cells proliferate and differentiate into two distinct major lineages: i) endocrine precursors expressing neurogenin3 (NGN3), and ii) pancreas specific transcription factor 1a (Ptf1a) exocrine progenitors, each with restricted differentiation potential. Each Neurog3-expressing cell is unipotent and several transcription factors, including Pdx1, Nkx6.1, Nkx2.2, Sox9, Ngn3, NeuroD1, Arx, Rfx6, Pax6, Mafa, and Mafb define the formation of each endocrine cell type along with NGN3. During development it appears that temporal modality determines the fate of Neurog3+ cells: the earliest Neurog3-expressing cells inclined to differentiate mainly into α-cells. The early endocrine cells accumulate into proto-islet-forming pools, whose size decides the final size of the islets [Heiker *et al.,* 2014; Brass *et al.,* 2004]. These initial developmental phases are vital because any change of the β-cell mass can result in defective blood glucose control, leading later in life to glucose intolerance and diabetes. Pancreas regeneration has been studied for >30 years using a multiplicity of rodent models which partly mimics either T1D or T2D, basically by reducing the β-cell mass and therefore impairing glucose homeostasis. Surgical techniques cause acute pancreatitis. Usually, diabetogenic partial PX (90% resection) and subdiabetogenic partial pancreatectomy (PX) (i.e. resection of 60–70% of the pancreas) infer the loss of a considerable portion of pancreatic cells, including β-cells, and can thus induce diabetes. Other surgical methods such as pancreatic main duct ligation, cellophane wrapping, and the administration of the drug caerulein, are protocols that induce acute pancreatitis, but do not reduce β-cell mass. After PX, on the contrary, there is an intense decrease of the β-cell mass, which is followed by a process of replacement or reconstitution (i.e. regeneration) of new β-cells. Induction of diabetes and β-cell regeneration are also detected in models of

chemically- or genetically-induced β-cell destruction. Considering the diverse methods and interpretations that have been reported it now appears that the mechanism and extent of β-cell mass increase could be varied and distinctive to each injury model. Three key regeneration processes have been described, namely: (i) neogenesis – undifferentiated precursor or progenitor cell differentiation to islet cells, (ii) replication of the remaining intact β-cells, and, more recently, (iii) transdifferentiation of pancreatic fully differentiated cells – conversion of one cell type into another (non-β cells to β-cells) by reprogramming (Figure 1.8) [Desgraz *et al.*, 2011; Vetere *et al.*, 2014].

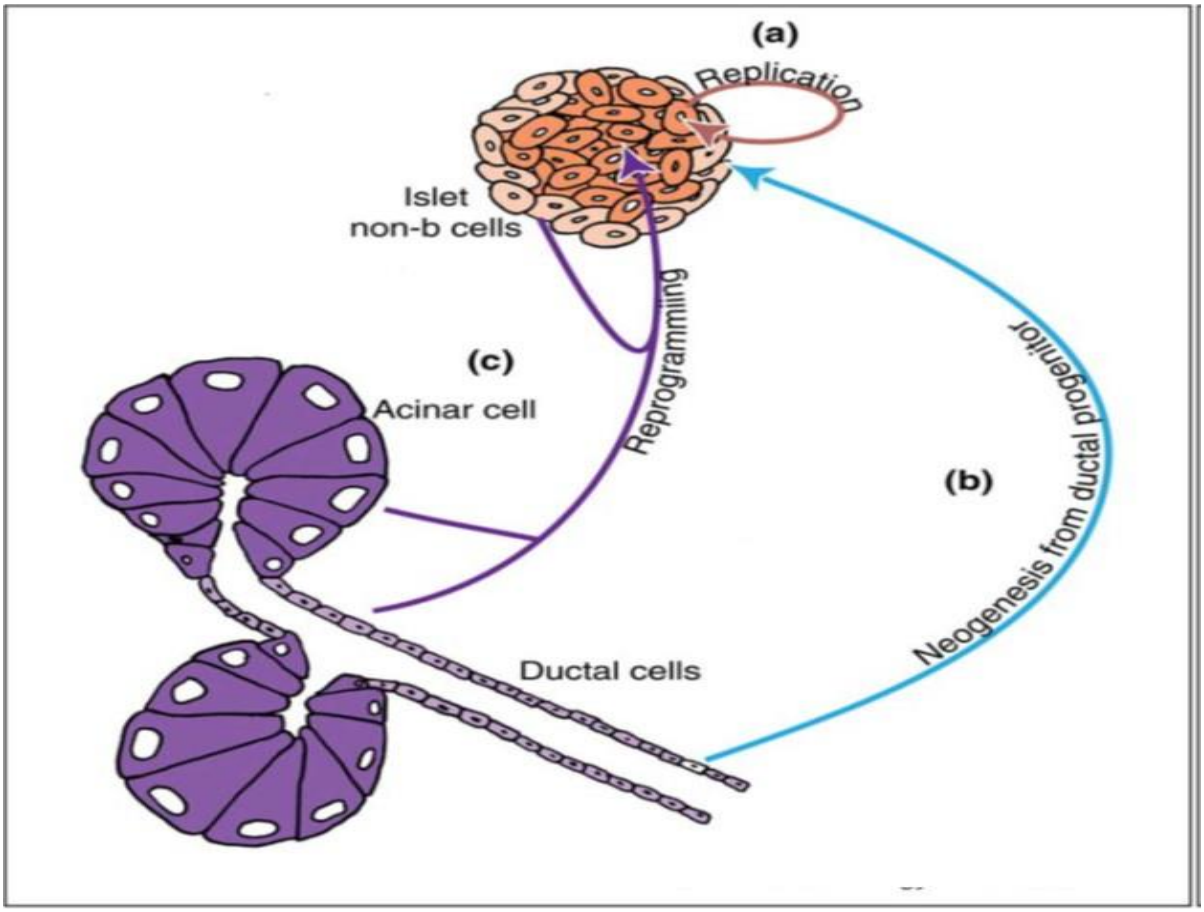

Figure 1.8 Cellular pathways to β-cell regeneration. According to the theories, three chief routes can give rise to new β-cells: (a) replication of remaining β-cells, (b) differentiation of undifferentiated precursors located in ducts or elsewhere, and (c) transdifferentiation (direct reprogramming) of fully differentiated pancreatic ductal, acinar or islet non-β-cells. Adapted from Desgraz *et al.*, 2011 [Desgraz *et al.*, 2011].

1.9 β-cell dysfunction/ death:

Reduced β-cell mass and function mark the clinical onset of diabetes mellitus. β-cell apoptosis in T2D is due to various factors, including obesity. Hypertrophy and hyperplasia of AT induced by macrophage infiltration cause the pathophysiology of obesity. Macrophages and inflamed adipocytes secrete inflammatory cytokines. It leads to the desensitisation of insulin-responsive tissues resulting in insulin resistance. Glucolipotoxicity and oxidative stress in β-cells are caused by persistent hyperglycemia and dyslipidemia. It endangers the β-cell integrity and functioning [Rathwa *et al.*, 2020]. The accumulation of FFA and DAG and the generation of high ROS levels add to both β-cell and adipocyte dysfunction. Their involvement in β-cell mitochondrial dysfunction is investigated. Insulin secretion from β-cells and adipokine secretion from the adipose tissues are both dependent on mitochondrial integrity. The excessive availability of nutrients hampers mitochondrial biogenesis. Mitochondrial dysfunction induces β-cell apoptosis and fatty liver disease. It stalls adipocyte differentiation and alters the balance of pro- vs anti-inflammatory adipokines (Figure 1.9) [Rathwa *et al.*, 2020].

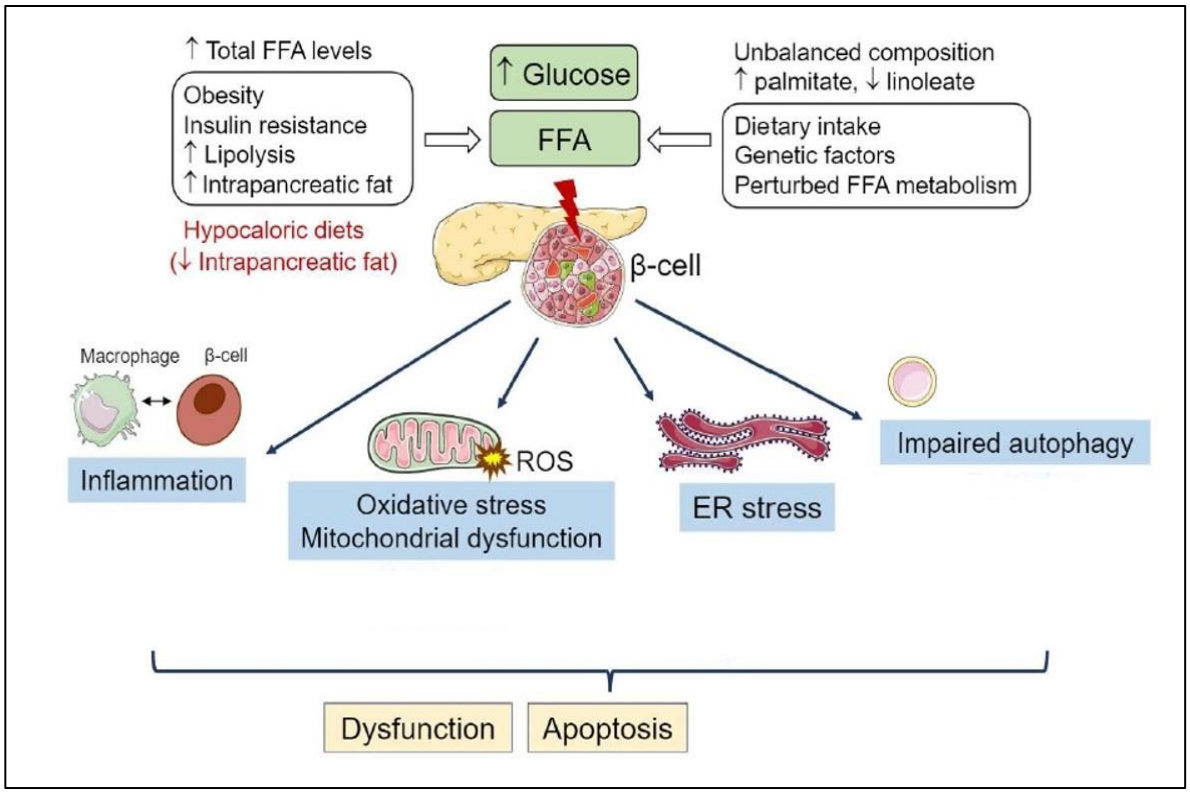

Figure 1.9 Molecular mechanisms of lipo- and glucolipotoxic β-cell apoptosis. A prolonged increased FFA and/or unbalanced FFA, alone or in combination with high glucose, elicit stress responses in pancreatic β-cells. These include ER stress, oxidative stress, mitochondrial dysfunction, inflammation and impaired autophagic flux. Crosstalk

between these pathways may induce downstream mechanisms that culminate in β-cell dysfunction and death. Adapted from Lytrivi *et al.*, 2020 [Lytrivi *et al.*, 2020].

1.10 Strategies to combat Diabetes Mellitus

The current treatments for T1D are insulin pumps, multiple-injection regimens, and insulin analogues though they often do not achieve the target glycated haemoglobin levels. Islet transplantation and stem cell therapy have long been proposed [JDRF, 2008]. The success of transplantation of islet cells showed restoration of normal blood glucose levels in diabetic rats for the first time >40 years ago [Kemp *et al.*, 1973; Miller *et al.*, 2015]. However, loss of islets during transplantation, islet death, anoxia, and engraftment are some of the reasons that decrease β-cell mass and induces early failure of the graft. Furthermore, the shortage of donor islets and cost-effectiveness are severe obstacles to the widespread application of islet transplantation as a cure. The pharmacological interventions for T2D involve two approaches: i) insulin secretion from β-cells and ii) insulin-mediated glucose uptake from peripheral tissues [Pramanik *et al.*, 2018]. The characteristics of the most widely used oral antidiabetic drugs are tabulated in Table 1.6 [Pramanik *et al.*, 2018]. The medication is prescribed based on the level of glycemic control required. When glycemic levels are high (e.g., A1C >8.5%), therapeutics with a rapid glucose-lowering capacity, or potentially earlier initiation of combination therapy are recommended. Similarly, when glycemic levels are closer to target goals (e.g., A1C <7.5%), diet/ diet and medications with lower hypoglycemic potential may be considered [Pramanik *et al.*, 2018].

Table 1.6: Characteristics of the most widely used oral antidiabetic drugs.

Group	Class	Generic name	Side effects
Biguanides	Sensitiser	Metformin	Weight loss, GI upset
Thiazolidinediones		Rosiglitazone Pioglitazone	Weight gain, Peripheral oedema
Alpha-glucosidase inhibitors	---	Acarbose Miglitol	GI upset
Sulfonylureas	Secretagogue	Chlorpropamide Glibenclamide Glimepiride	Hypoglycemia Weight gain

		Glipizide Tolazamide Tolbutamide	
Glinides		Nateglinide Repaglinide	Weight gain
Exenatide	GLP-1 analog	Byetta	Weight loss
Dipeptidyl peptidase-4 inhibitors DPP-IV)	Inhibits DPP-IV enzyme, which prolongs the action of the incretin hor mones. It promotes insulin secretion and inhibits glucagon secretion.	Sitagliptin, Saxagliptin, Linagliptin, Vildagliptin Alogliptin	GI problems, Flu-like symptoms
GLP-1 receptor agonists	Incretin mimetics. It promotes insulin secretion and inhibits glucagon secretion.	Exenatide Liraglutide Semaglutide Dulaglutide Lixisenatide	Nausea, GI upset, Headache
SGLT2 inhibitors	Reduces renal tubular glucose reabsorption.	Canagliflozin	Urinary tract infection, Bone fractures, Nausea

However, patients develop tolerance against these drugs within a few years of treatment, which poses a challenge for developing new medicines. Since control of glucose levels can thwart the devastating complications of diabetes, research now focuses on β-cell replacement therapy, which can be accomplished by regenerating the deficient β-cells in the pancreas. Therefore, the focus is on the cost-effective therapeutic strategies to preserve or expand the β-cell mass and function for DM. However, it is a perplexing task because β-cells do not undergo regeneration in the adult phase and are considered quiescent cell type compared to hepatocytes [Meier *et al.,* 2008]. Moreover, the challenge for the T2D is to maintain glycemic control and discover drugs targeting multiple diabetes-related complications. Another problem is that we are still not fully aware of human β-cell proliferation and their regulatory intricacies and it is complicated to understand this due to the different experimental models used for these studies [Bernal-Mizrachi *et al.,* 2014]. Hence, more research is getting orientated towards identifying the molecules involved and their modes of action towards β-cell regeneration**.**

1.10.1 Contemporary therapies having the power of β-cell regeneration

The first line of treatment involves nutrition cut-down and enhanced physical activity for the borderline to early-stage T2D. When the desired glycemic control is unachievable, the patient is prescribed medicines like metformin, sulfonylureas and insulin to lower glucose, that work through different mechanisms. However, due to unknown reasons, most of them lose their efficacy with time, resulting in progressive β-cell worsening. β-cell mass is the total weight of β-cells within the pancreas. It is regulated by the equilibrium between formation (replication of existing cells and neogenesis/transdifferentiation) and death (apoptosis/necrosis) of β-cells as well as individual cell volume (atrophy/hypertrophy) [Desgraz *et al.,* 2011; Aguayo-Mazzucato *et al.,* 2018]. The β-cell function is the quantitative correlation between insulin sensitivity and insulin action to adapt and sense healthy individual's metabolic environment [Lorenzo *et al.,* 1994].

1.11 Diet: acting as a switch to regulate Type 2 Diabetes

Dietary intervention has long been considered first-line therapy for diabetes management by researchers and clinicians worldwide. Interestingly, numerous reports emphasise the benefits of tailor-made diets on insulin signalling pathways and β-cell functionality, i.e., calorie restriction (CR). CR reduces calorie intake without cutting down on vital nutrients [Rathwa et al., 2020, Sohal *et al.,* 1996]. CR was proposed and studied as a strategy for

increasing the longevity of life by attenuation of oxidative stress [Sohal *et al.,* 1996] and promoting mitochondrial function and biogenesis through the expression of peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1α), transcription factor A, Mitochondrial (TFAM), and Sirtuin 1 (SIRT1) [Civitarese *et al.,* 2007]. The elevated rate of whole-body fat oxidation in response to CR was observed, along with reduced levels of FFA synthesis in the liver [Lytrivi *et al.,* 2020]. Improved FBG offers protection from other cardiometabolic risk factors and reduces pro-inflammatory adipokines [Pramanik *et al.,* 2018; Rathwa *et al.,* 2020]. The relationship between the phosphorylation (and therefore activation) of ERK and p70S6K along with the phosphorylation of IRS1S612 and IRS1S632/635 implicates ERK and MTOR/ p70S6K as the kinases responsible for the phosphorylation of these sites in the liver as observed in obesity-induced insulin resistance. However, CR diminished the activities of these kinases, ameliorating insulin resistance [Pramanik *et al.,* 2018; Rathwa *et al.,* 2020].

Nevertheless, there are a few reports on β-cell regeneration by CR. Cheng *et al.* have shown the outcome of a fast-mimicking diet on NGN3 and Pdx-1, the major β-cell-mediated transcription factor. The authors showed that in human T1D pancreatic islets, fasting situations lessen PKA and mTOR activity while promoting Sox2, NGN3, and insulin expression [Cheng *et al.,* 2017]. A new strategy for time-restricted diet has been proposed and studied. It showed that pro-inflammatory markers influence the circadian rhythm and enhance the β-cell responsiveness in prediabetic men [Rathwa *et al.,* 2020]. However, several reports suggest that dietary intervention in T2D progression by reversing insulin resistance and restoring β-cell functionality does not affect β-cell regeneration [Pramanik *et al.,* 2018; Rathwa *et al.,* 2020]. Although the above studies are encouraging for T2D management, it is essential to curb β-cell loss in T1D and T2D. Thus, limitations of dietary approaches drive the need for alternative strategies.

1.12 γ-Aminobutyric Acid (GABA): Potential β-cell regeneration molecule

GABA has emerged as a new anti-diabetic dietary supplement. GABA, a major inhibitory neurotransmitter, has proven to have a role in islet-cell hormone homeostasis, preservation of the β-cell mass, suppressing detrimental immune reactions and apoptosis [Soltani *et al.,* 2011; Prud'homme *et al.,* 2013; Wang *et al.,* 2019]. L-glutamate is decarboxylated to GABA by any of two glutamic acid decarboxylase isoforms (GAD 65 and GAD 67). In neurons, GAD65 is found in synaptic vesicles, whereas GAD67 has a cytosolic location [Rathwa *et al.,* 2020; Wang *et al.,* 2019]. Pancreatic expression of GAD in β-cells differs between

humans and mice. In humans, GAD65 is predominant, while in mice it is GAD67 [Wang *et al.*, 2019]. It is known that GAD 65 antibodies are one of the most prevalent markers of β-cell destruction in type-1 diabetes. GABA exerts a negative autocrine and paracrine control of insulin and glucagon secretion, respectively, by acting on specific GABA receptors on the pancreatic islets. GABA receptors are classified as Type A ($GABA_AR$) and Type B ($GABA_BR$). These receptors are present on the membrane of both β- and α-cells [Wan *et al.*, 2015], and are quite diverse in their physiological and molecular characteristics. $GABA_ARs$ are ligand-gated Cl^- ion channels [Rudolp *et al.*, 2011], that are typically arranged as heteropentamers. Several $GABA_AR$ subunits (α 1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3) have been identified [Wang *et al.*, 2019]. The functional GABA receptor typically consists of two α subunits, two β subunits and one other subunit [Wang *et al.*, 2019]. Human β-cell $GABA_ARs$ are generally formed by combinations of the various α chains, β3, and γ2 subunits [Wang *et al.*, 2019]. GABA appears to exert stimulatory effects on β-cell insulin secretion via a mechanism involving membrane depolarisation and a VGCC-dependent Ca^{2+} movement [Wang *et al.*, 2019]. In isolated human islets, GABA-induced activation of PI3K/Akt pathway is mediated mainly by $GABA_AR$, and partially by $GABA_BR$ [Purwana *et al.*, 2014]. It also stimulates the phosphorylation of cAMP response element-binding protein (CREB) [Wang *et al.*, 2019], known as a key transcription factor responsible for the maintenance of insulin gene transcription and β-cell survival in rodent and human islets. The regular control of secretion of insulin and glucagon from pancreatic islets is dynamic for the maintenance of blood glucose homeostasis and is regulated by GABA, which can be defective in T2D condition (Figure 1.10) [Rutter *et al.*, 2019]. The promising effects of GABA action have been reported in T1D and T2D murine models. There are several preclinical/clinical studies reported on β-cell proliferation effected by GABA and GABA receptor agonist (Table 1.7) [Wang *et al.*, 2019]. GABA therapy protects NOD mice from diabetes [Soltani *et al.*, 2011; Liu *et al.*, 2017]. Also, GABA generally regulates cytokine secretion from human PBMCs and suppresses β-cell-reactive CD8+ CTLs in T1D models [Rathwa *et al.*, 2020; Soltani *et al.*, 2011; Wang *et al.*, 2019]. The proposed role of GABA as an immunosuppressant is thus considered. GABA may act as an inducer of α-to-β-like cell conversion *in-vivo* upon prolonged exposure in the STZ-induced mouse model [Ben-Othman *et al.*, 2017]. The encouraging reports of GABA on T1D led to studies on T2D models as well. GABA has been reported to promote human β-cell replication and islet cell survival in *in-vivo* and humanised mice. Combined GABA and sitagliptin (DPP-IV inhibitor) therapy have demonstrated β-cell regenerative effects in diabetic mouse models

[Liu *et al.*, 2017; Zhong *et al.*, 2019]. The up-regulation of Pdx-1 expression contributes to β-cell replication. All these data indicate the therapeutic potential of GABA to stimulate the growth and function of insulin-producing β-cells and act as an immunosuppressive agent in diabetic therapy (Table 7) [Wang *et al.*, 2019].

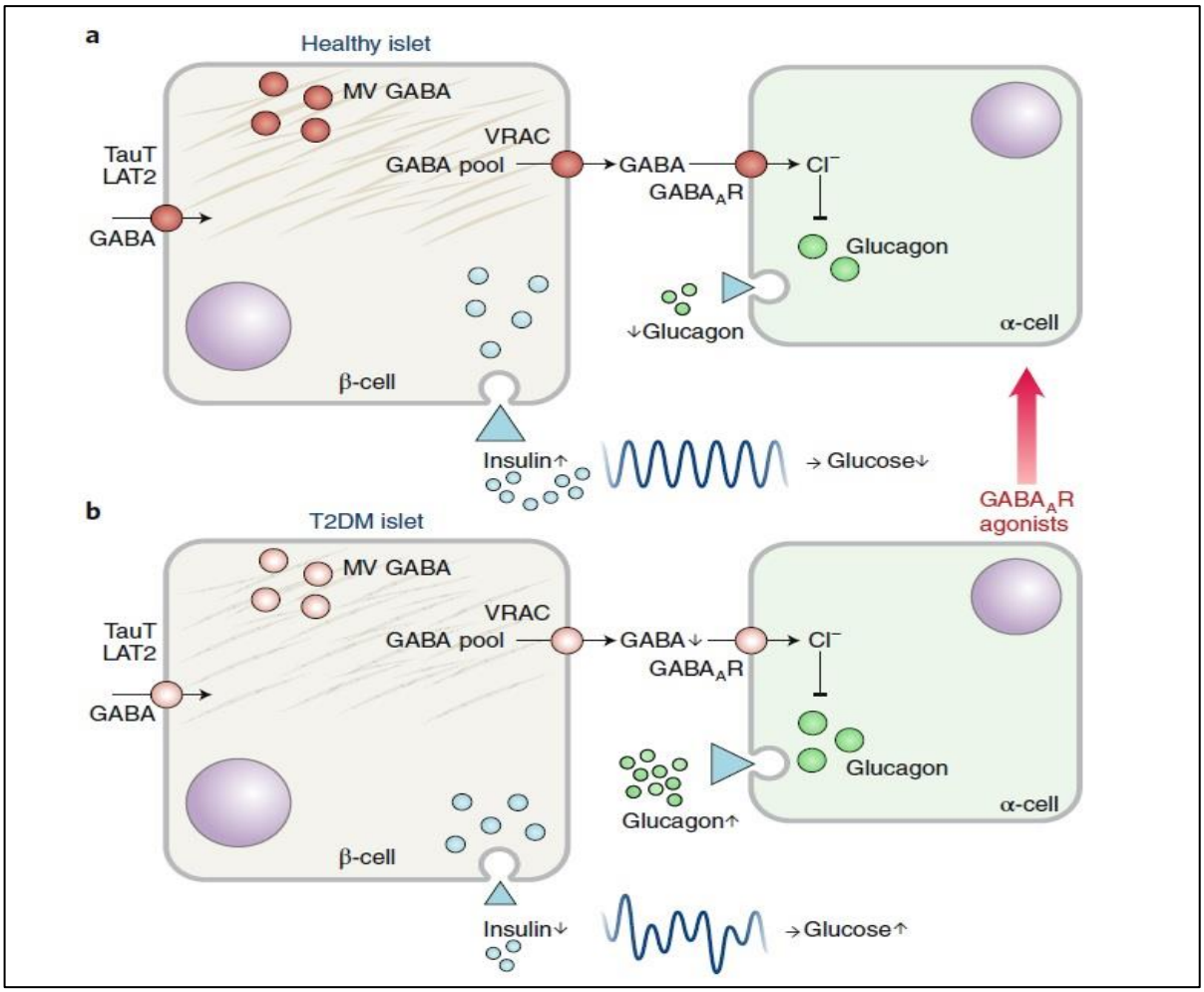

Figure 1.10 The functions of GABA in pancreatic cells, in health and in T2D. A cytosolic pool of GABA in most β-cells permits constitutive secretion of the neurotransmitter, thus suppressing both insulin release (autocrine) and glucagon secretion from α-cells (paracrine). GABA release from somatostatin-expressing δ-cells may also contribute to the extracellular pool of GABA. In healthy individuals, constitutive GABA secretion from most β-cells confirms regular oscillations in insulin secretion and suppresses glucagon secretion, thus ensuring effective glucose regulation. In T2D patients, suppressed GABA de-inhibits glucagon secretion and may contribute to erratic oscillations in insulin secretion. MV, microvesicle; $GABA_AR$, $GABA_A$ receptor. Adapted from Rutter *et al.*, 2019 [73].

Table 1.7: Stimulation of β-cell proliferation by GABA or GABA-receptor agonist.

		Percent of β cells replicating		
Agonist	Cell type and treatment	Basal	GABAR agonist†	Reference
GABA	Rat islet (in vitro)	1.25 BrdU	2.25 BrdU (Ins ↑)‡	Ligon et al. (2007)
GABA	Mouse islet (in vivo, MLD-STZ)	0.4 BrdU	1.1 BrdU	Soltani et al. (2011)
		0.3 Ki67	1.2 Ki67 (Ins ↑)	
GABA	Mouse islet (in vivo, MLD-STZ)	0.4 Ki-67	1.2 Ki67 (Ins ↑)	Liu et al. (2017)
GABA	Mouse islet (in vivo, partial pancreatectomy)	0.5 BrdU	2.2 BrdU	Wang et al. (2013)
GABA	Mouse islet (in vivo, NOD mouse)	<0.05 Ki67	1.2 Ki67	Tian et al. (2014)
GABA	Human islet (in vivo, xenotransplantation)	0.8 BrdU	1.9 BrdU	Tian et al. (2013)
		0.4 Ki67	0.9 Ki67	
GABA	Human islet (in vivo, xenotransplantation)	0.5 BrdU	2.2 BrdU (Ins ↑)	Purwana et al. (2014)
	Human islet (in vitro)	0.7 Ki67	1.8 Ki67	
	Human islet (in vitro)	0.1 BrdU	0.3 BrdU (Ins ↑)	
$GABA_BR$ agonist	Human islet (in vivo, xenotransplantation)	0.8 BrdU	1.9 BrdU	Tian et al. (2017a)
		0.5 Ki67	0.9 Ki67	
GABA	Human islet (in vitro)	0.03 Ki67	0.1 Ki67	Aamodt et al. (2016)
GABA	CD1 mice (in vivo, normoglycemic mice)	$6E^{-7}$ Ki67	$9.5E^{-7}$ Ki67§	Untereiner et al. (2018)
	CD1 islet (in vitro)	0.84 Ki67	3.8 Ki67§	
	Human islet (in vitro)	0.6 Ki67	1.5 Ki67§	

GABAR: γ-aminobutyric acid receptor; MLD-STZ: multiple low-dose streptozotocin model of diabetes; NOD: nonobese diabetic; Ins+: insulin-positive cells. †GABAR agonist: *In vitro* or *in vivo* application of a GABA receptor agonist. Some values were estimated from published Figures. All the results listed with a GABAR agonist were statistically significant as compared to the basal (unstimulated) replication rate. ‡Ins ↑, GABA treatment increased insulin secretion *in vitro* or *in vivo*. §In this study only, results were expressed as the percent of Ins+/Ki67+ cells in the total islet-cell population. α-cell numbers were also increased. GABA increased insulin *in vivo*, but not *in vitro*. In humans, results are the mean of six out of nine donors showing GABA-induced proliferation, the β cells of three donors did not respond to GABA. In some human donors, the proliferation induced by GABA was comparable or superior to stimulation with Harmine (a mitogenic agent that induces β-cell proliferation).

References:

1. Atlas D. International diabetes federation. IDF Diabetes Atlas, 9th edn. Brussels, Belgium: International Diabetes Federation. 2019
2. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, Adhikari P, Rao PV, Saboo B, Kumar A, Bhansali A. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR–INDIAB population-based cross-sectional study. The lancet Diabetes & endocrinology. 2017 1;5(8):585-96.
3. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, Nath LM, Das AK, Madhu V, Rao PV, Shukla DK. The Indian Council of Medical Research—India Diabetes (ICMR-INDIAB) Study: Methodological Details. Journal of diabetes science and technology. 2011;5(4):906-14.
4. Anjana RM, Kesavadev J, Neeta D, Tiwaskar M, Pradeepa R, Jebarani S, Thangamani S, Sastry NG, Brijendra Kumar S, Ramu M, Gupta PP. A multicenter real-life study on the effect of flash glucose monitoring on glycemic control in patients with type 1 and type 2 diabetes. Diabetes technology & therapeutics. 2017 ;19(9):533-40.
5. Aguayo-Mazzucato C, Bonner-Weir S. Pancreatic β cell regeneration as a possible therapy for diabetes. Cell metabolism. 2018;27(1):57-67.
6. Bastidas-Ponce A, Scheibner K, Lickert H, Bakhti M. Cellular and molecular mechanisms coordinating pancreas development. Development. 2017;144(16):2873-88.
7. Ben-Othman N, Vieira A, Courtney M, Record F, Gjernes E, Avolio F, Hadzic B, Druelle N, Napolitano T, Navarro-Sanz S, Silvano S. Long-term GABA administration induces alpha cell-mediated beta-like cell neogenesis. Cell. 2017;168(1-2):73-85.
8. Bernal-Mizrachi E, Kulkarni RN, Scott DK, Mauvais-Jarvis F, Stewart AF, Garcia-Ocaña A. Human β-cell proliferation and intracellular signaling part 2: still driving in the dark without a road map. Diabetes. 2014;63(3):819-31.
9. Brass BJ. Endocrine pancreas: mechanisms of insulin secretion by the β-cells; interactions among insulin, glucagon and somatostatin. InPrinciples of Diabetes Mellitus 2004 (pp. 57-78). Springer, Boston, MA.
10. Bonner-Weir S, Guo L, Li WC, Ouziel-Yahalom L, Lysy PA, Weir GC, Sharma A. Islet neogenesis: a possible pathway for beta-cell replenishment. The review of diabetic studies: RDS. 2012;9(4):407.

11. Cheng CW, Villani V, Buono R, Wei M, Kumar S, Yilmaz OH, Cohen P, Sneddon JB, Perin L, Longo VD. Fasting-mimicking diet promotes Ngn3-driven β-cell regeneration to reverse diabetes. Cell. 2017;168(5):775-88.
12. Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, Smith SR, Ravussin E. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS med. 2007;4(3):e76.
13. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nature medicine. 2017;23(7):804.
14. Da Silva Xavier G. The cells of the islets of Langerhans. Journal of clinical medicine. 2018;7(3):54.
15. Desgraz R, Bonal C, Herrera PL. β-cell regeneration: the pancreatic intrinsic faculty. Trends in Endocrinology & Metabolism. 2011;22(1):34-43.
16. Dwivedi M, Laddha NC, Imran M, Ansarullah, Bajpai P, Ramachandran AV, Misra A, Yadav M, Begum R. ACE gene I/D polymorphism in type 2 diabetes: the Gujarat population. The British Journal of Diabetes & Vascular Disease. 2011;11(3):153-4.
17. Fain JN, Cheema PS, Bahouth SW, Hiler ML. Resistin release by human adipose tissue explants in primary culture. Biochemical and biophysical research communications. 2003;300(3):674-8.
18. Filková M, Haluzík M, Gay S, Šenolt L. The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clinical immunology. 2009;133(2):157-70.
19. Fu Z, R Gilbert E, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Current diabetes reviews. 2013;9(1):25-53.
20. Gao R, Ustinov J, Pulkkinen MA, Lundin K, Korsgren O, Otonkoski T. Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes. 2003;52(8):2007-15.
21. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annual review of immunology. 2011;23;29:415-45.
22. Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian journal of diabetes. 2013;37:S8-11.
23. Heiker JT. Vaspin (serpinA12) in obesity, insulin resistance, and inflammation. Journal of Peptide Science. 2014;20(5):299-306.

24. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proceedings of the national academy of sciences. 2005;102(30):10610-5.
25. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obesity research. 2002;10(1):1-5.
26. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. New England Journal of Medicine. 2008;359(14):1464-76.
27. Kearney J. Food consumption trends and drivers. Philosophical transactions of the royal society B: biological sciences. 2010;365(1554):2793-807.
28. Kempf K, Rose B, Illig T, Rathmann W, Strassburger K, Thorand B, Meisinger C, Wichmann HE, Herder C, Vollmert C. Vaspin (SERPINA12) genotypes and risk of type 2 diabetes: results from the MONICA/KORA studies. Experimental and clinical endocrinology & diabetes. 2010;118(03):184-9.
29. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia. 1973;9(6):486-91.
30. Kirsch I. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. Preface. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2011;366(1572):1781-2.
31. Koria B, Kumar R, Nayak A, Kedia G. Prevalence of diabetes mellitus in urban population of Ahmadabad city, Gujarat. Natl J Community Med. 2013;4(3):398-401.
32. Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, Lee S, Kim JY, Lee J, Yang HM, Mook-Jung I. Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. Cell metabolism. 2014;19(3):484-97.
33. Lieberman SM, Evans AM, Han B, Takaki T, Vinnitskaya Y, Caldwell JA, Serreze DV, Shabanowitz J, Hunt DF, Nathenson SG, Santamaria P. Identification of the β cell antigen targeted by a prevalent population of pathogenic CD8+ T cells in autoimmune diabetes. Proceedings of the National Academy of Sciences. 2003;100(14):8384-8.

34. Liu W, Son DO, Lau HK, Zhou Y, Prud'homme GJ, Jin T, Wang Q. Combined oral administration of GABA and DPP-4 inhibitor prevents beta cell damage and promotes beta cell regeneration in mice. Frontiers in pharmacology. 2017;8:362.
35. Lu SC, Shieh WY, Chen CY, Hsu SC, Chen HL. Lipopolysaccharide increases resistin gene expression in vivo and in vitro. FEBS letters. 2002;530(1-3):158-62.
36. Lytrivi M, Castell AL, Poitout V, Cnop M. Recent insights into mechanisms of β-cell lipo-and glucolipotoxicity in type 2 diabetes. Journal of molecular biology. 2020;432(5):1514-34.
37. Lorenzo A, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature. 1994;368(6473):756-60.
38. Mellitus D. Diagnosis and classification of diabetes mellitus. Diabetes care. 2005;28(S37):S5-10.
39. Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, Rizza RA, Butler PC. β-Cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. Diabetes. 2008;57(6):1584-94.
40. Miller KM, Foster NC, Beck RW, Bergenstal RM, DuBose SN, DiMeglio LA, Maahs DM, Tamborlane WV. Current state of type 1 diabetes treatment in the US: updated data from the T1D Exchange clinic registry. Diabetes care. 2015;38(6):971-8.
41. Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochemical and biophysical research communications. 2001;285(2):561-4.
42. Nakatsuka A, Wada J, Iseda I, Teshigawara S, Higashio K, Murakami K, Kanzaki M, Inoue K, Terami T, Katayama A, Hida K. Vaspin is an adipokine ameliorating ER stress in obesity as a ligand for cell-surface GRP78/MTJ-1 complex. Diabetes. 2012;61(11):2823-32.
43. Nakatsuka A, Wada J, Iseda I, Teshigawara S, Higashio K, Murakami K, Kanzaki M, Inoue K, Terami T, Katayama A, Hida K. Visceral adipose tissue-derived serine proteinase inhibitor inhibits apoptosis of endothelial cells as a ligand for the cell-surface GRP78/voltage-dependent anion channel complex. Circulation Research. 2013;112(5):771-80.
44. Ohashi K, Shibata R, Murohara T, Ouchi N. Role of anti-inflammatory adipokines in obesity-related diseases. Trends in endocrinology & metabolism. 2014;25(7):348-55.

45. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovascular diabetology. 2018;17(1):1-4.
46. Patel R, Dwivedi M, Mansuri MS, Laddha NC, Thakker A, Ramachandran AV, Begum R. Association of neuropeptide-Y (NPY) and interleukin-1beta (IL1B), genotype-phenotype correlation and plasma lipids with Type-II diabetes. PloS one. 2016;11(10):e0164437.
47. Patel R, Palit SP, Rathwa N, Ramachandran AV, Begum R. Genetic variants of tumor necrosis factor-α and its levels: A correlation with dyslipidemia and type 2 diabetes susceptibility. Clinical nutrition. 2019;38(3):1414-22.
48. Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family hormones. Science. 2004;304(5674):1154-8.
49. Palit SP, Patel R, Jadeja SD, Rathwa N, Mahajan A, Ramachandran AV, Dhar MK, Sharma S, Begum R. A genetic analysis identifies a haplotype at adiponectin locus: association with obesity and type 2 diabetes. Scientific reports. 2020;10(1):1-0.
50. Pessin JE, Kwon H. Adipokines mediate inflammation and insulin resistance. Frontiers in endocrinology. 2013;4:71.
51. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutrition reviews. 2012;70(1):3-21.
52. Pramanik S, Rathwa N, Patel R, Ramachandran AV, Begum R. Treatment avenues for type 2 diabetes and current perspectives on adipokines. Current diabetes reviews. 2018;14(3):201-21.
53. Prud'homme GJ, Glinka Y, Hasilo C, Paraskevas S, Li X, Wang Q. GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone. Transplantation. 2013;96(7):616-23.
54. Purwana I, Zheng J, Li X, Deurloo M, Son DO, Zhang Z, Liang C, Shen E, Tadkase A, Feng ZP, Li Y. GABA promotes human β-cell proliferation and modulates glucose homeostasis. Diabetes. 2014;63(12):4197-205.
55. Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule–β selectively impair insulin action on glucose production. The Journal of clinical investigation. 2003;111(2):225-30.
56. Rangwala SM, Rich AS, Rhoades B, Shapiro JS, Obici S, Rossetti L, Lazar MA. Abnormal glucose homeostasis due to chronic hyperresistinemia. Diabetes. 2004;53(8):1937-41.

57. Rathwa N, Patel R, Palit SP, Parmar N, Rana S, Ansari MI, Ramachandran AV, Begum R. β-cell replenishment: Possible curative approaches for diabetes mellitus. Nutrition, Metabolism and Cardiovascular Diseases. 2020;30(11):1870-81.
58. Rathwa N, Patel R, Palit SP, Ramachandran AV, Begum R. Genetic variants of resistin and its plasma levels: Association with obesity and dyslipidemia related to type 2 diabetes susceptibility. Genomics. 2019;111(4):980-5.
59. Rathwa N, Patel R, Palit SP, Jadeja SD, Narwaria M, Ramachandran AV, Begum R. Circulatory Omentin-1 levels but not genetic variants influence the pathophysiology of Type 2 diabetes. Cytokine. 2019;119:144-51.
60. Rathwa N, Parmar N, Palit SP, Patel R, Ramachandran AV, Begum R. Intron specific polymorphic site of vaspin gene along with vaspin circulatory levels can influence pathophysiology of type 2 diabetes. Life sciences. 2020;243:117285.
61. Rotella CM, Pala L, Mannucci E. Role of insulin in the type 2 diabetes therapy: past, present and future. International journal of endocrinology and metabolism. 2013;11(3):137.
62. Rudolph U, Knoflach F. Beyond classical benzodiazepines: novel therapeutic potential of GABA A receptor subtypes. Nature reviews Drug discovery. 2011 ;10(9):685-97.
63. Rutter GA. Pancreatic islet secretion: gabbling via GABA. Nature Metabolism. 2019 ;1(11):1032-3.
64. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852-71.
65. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF-α and IL-12 in macrophages by NF-κB-dependent pathway. Biochemical and biophysical research communications. 2005;334(4):1092-101.
66. Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, Li Y, Zhang N, Chakrabarti R, Ng T, Jin T. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. Proceedings of the National Academy of Sciences. 2011;108(28):11692-7.
67. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996;273(5271):59-63.

68. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. Nature. 2001;409(6818):307-12.
69. Steppan CM, Wang J, Whiteman EL, Birnbaum MJ, Lazar MA. Activation of SOCS-3 by resistin. Molecular and cellular biology. 2005;25(4):1569.
70. Szpigel A, Hainault I, Carlier A, Venteclef N, Batto AF, Hajduch E, Bernard C, Ktorza A, Gautier JF, Ferré P, Bourron O. Lipid environment induces ER stress, TXNIP expression and inflammation in immune cells of individuals with type 2 diabetes. Diabetologia. 2018;61(2):399-412.
71. Tomita T. Apoptosis of pancreatic β-cells in type 1 diabetes. Bosnian journal of basic medical sciences. 2017;17(3):183.
72. Vetere A, Choudhary A, Burns SM, Wagner BK. Targeting the pancreatic β-cell to treat diabetes. Nature reviews Drug discovery. 2014;13(4):278-89.
73. Wang Q, Ren L, Wan Y, Prud'homme GJ. GABAergic regulation of pancreatic islet cells: physiology and antidiabetic effects. Journal of cellular physiology. 2019;234(9):14432-44.
74. Wan Y, Wang Q, Prud'homme GJ. GABAergic system in the endocrine pancreas: a new target for diabetes treatment. Diabetes, metabolic syndrome and obesity: targets and therapy. 2015;8:79.
75. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC, Gong DW. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. American journal of physiology-endocrinology and metabolism. 2006;290(6):E1253-61.
76. Zhong F, Jiang Y. Endogenous pancreatic β cell regeneration: A potential strategy for the recovery of β cell deficiency in diabetes. Frontiers in endocrinology. 2019;10:101.

OBJECTIVES

Objectives:

Objective I: To study the role of Resistin in T2D.

a. To study association of the following *resistin* gene polymorphisms with T2D in Gujarat population:

i. -420 C/G (rs1862513) ii. -358 G/A (rs3219175) iii. -638 C/G (rs34861192)

b. To assess plasma resistin protein levels.

c. To perform a possible genotype-phenotype correlation analysis with metabolic parameters and T2D susceptibility.

Objective II: To study the role of Omentin-1 in T2D.

a. To study association of the following *omentin-1* gene polymorphisms with T2D in Gujarat population:

i. Intron 1 G/T (rs1333062) ii. Exon 4 Val109Asp (rs2274907)

b. To assess plasma omentin-1 protein levels and *omentin-1* transcript levels in adipose tissue.

c. To perform a possible genotype-phenotype correlation analysis with metabolic parameters and T2D susceptibility.

Objective III: To study the role of Vaspin in T2D.

a. To study association of the following *vaspin* gene polymorphisms with T2D in Gujarat population:

i. Intron 1 A/G (rs76624128) ii. Intron 2 G/T (rs77060950)

b. To assess plasma vaspin protein levels and *vaspin* transcript levels in adipose tissue.

c. To perform a possible genotype-phenotype correlation analysis with metabolic parameters and T2D susceptibility.

Objective IV: To investigate the effect of γ-Aminobutyric acid (GABA), Calorie Restriction (CR) and combination treatment on pancreatic β-cell proliferation in High-Fat Diet (HFD) + Streptozotocin (STZ) induced experimental mouse model.

a. To establish HFD + STZ induced T2D mouse model.

b. To assess glucose tolerance and insulin sensitivity.

c. To estimate plasma lipid profile, insulin and c-peptide levels.

d. To study transcript levels of glucoregulatory enzymes in the liver; lipid metabolism enzymes in the adipose tissue and mitochondrial biogenesis markers in the skeletal muscle.

e. To study mitochondrial respiration in the skeletal muscle.

f. To assess β-cell regeneration and apoptosis in the pancreas.