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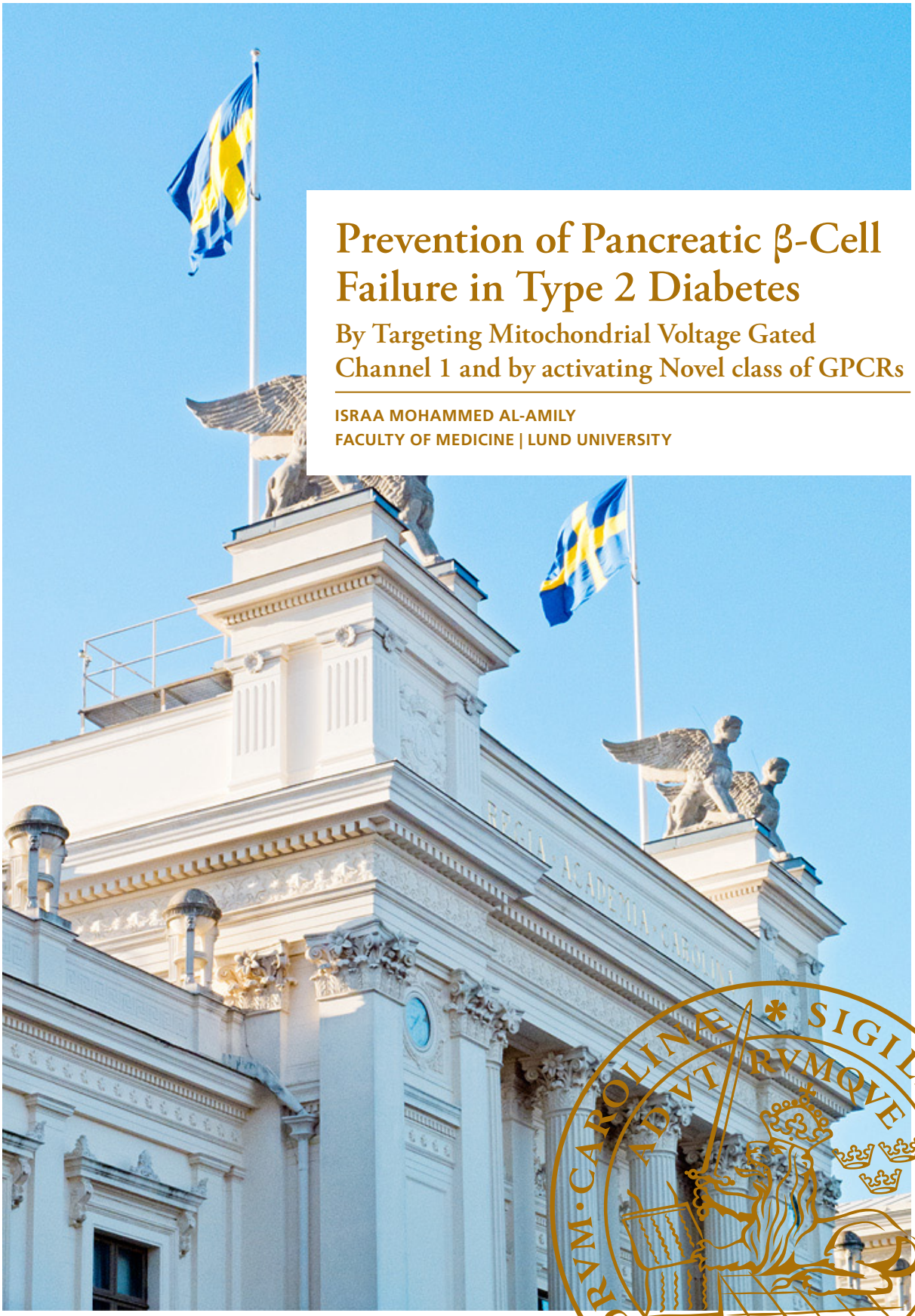

Prevention of Pancreatic β-Cell Failure in Type 2 Diabetes

By Targeting Mitochondrial Voltage Gated Channel 1 and by activating Novel class of GPCRs

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FACULTY OF MEDICINE | LUND UNIVERSITY

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Abstract

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It has been long known that hyperglycaemia-induced β-cell dysfunction precipitates type 2 diabetes (T2D) in insulin-resistant obesity, although the underlying mechanisms are still poorly defined. The few frequently used antidiabetic drugs on the market have still not satisfactorily demonstrated any long-lasting improvements of β-cell function and prevention of the disease. Islets synthesise and secrete numerous peptides, many of which having important impact on the regulation of metabolism, in particular blood glucose control. In paper I, we quantified mRNAs encoding all peptide ligands of islet G protein-coupled receptors (GPCRs) in isolated human and mouse islets. The study will allow accurate translation of mouse islet functional studies relevant for human physiology, which may pave the way to novel treatment of diabetes. In paper II, we show that ADGRG1 (GPR56) is the most abundant GPCR transcript in both human and mouse islets, and its expression in human islets strongly correlates with genes important for β-cell function. ADGRG1 was reduced in islets of T2D donors, in *db/db* mouse islets, and in isolated human non-diabetic islets exposed to chronic hyperglycaemia (high glucose concentration in vitro). ADGRG1 activation increased cAMP generation and exerted ant-apoptotic effects. In paper III, we show that long-lasting exposure to high glucose (glucotoxicity) impairs ATP production in β-cells, due to overexpression and coll of the mitochondrial membrane protein VDAC1 to the cell surface. This causes ATP loss, whose attenuation with VDAC1 inhibitors restores β-cell function. Daily injections of a VDAC1 inhibitor prevent the onset of hyperglycaemia in *db/db* mice. Thus, β-cell function is preserved by targeting the novel diabetes executer protein VDAC1.

In paper IV, we show that Adgrg1/GPR56 knock-down (KD) in mouse islets increases the activity of P70S6K, JNK, AKT, NFkB, STAT3 and STAT5. Similar to the hyperglycaemia-induced β-cell dysfunction, the Adgrg1-KD induced β-cell dysfunction seems to be associated with translocation of mitochondrial Vdac1 to the cell membrane resulting in an increased loss of cellular ATP.

In paper V, we studied the role of GPR142, expressed in both mouse and human islets. Gpr142 activation by specific agonists increases the generation of cAMP in mouse islets and long-term exposure to high glucose also reduces the expression of Gpr142. Gpr142-KD was associated with increase in Vdac1 expression in mouse islets and INS-1 cells.

Taken together the information presented in the current thesis identified the involvement of the novel diabetes executer protein VDAC1 in β-cell dysfunction and suggests VDAC1 as a new target for the prevention of T2D. Keeping the heterogeneity of T2D in mind, additional opportunities *i.e.* targeting two newly deorphanized GPCRs *i.e.* ADGRG1 (GPR56) and GPR142 has also been highlighted as novel therapies for the prevention of human T2D.

Key words: Type 2 diabetes, insulin, β-cell dysfunction, GPCRs, VDAC, ATP, cAMP, glucotoxicity.

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To my family,

You have enriched my life.

Discovery is seeing what everybody else has seen and thinking what nobody else has thought. Albert Szent-Györgyi

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Adhesion G-protein coupled receptor G1 (ADGRG1/GPR56) and pancreatic β-cell function.
Pontus Dunér*, **Israa Mohammad Al-Amily***, Arvind Soni, Olof Asplund, Fateme Safi, Petter Storm, Leif Groop, Stefan Amisten, and Albert Salehi. J Clin Endocrinol Metab 2016 Dec;101(12):4637-4645.
*Equal contribution

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Paper IV

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Amisten S, **Mohammad Al-Amily I**, Soni A, Hawkes R, Atanes P, Persaud SJ, Rorsman P, Salehi A. Endocr J. 2017;64(3):325-338.

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Activation of imidazoline receptor I2, and improved pancreatic β-cell function in human islets.
Amisten S, Duner P, Asplund O, **Mohammed Al-Amily I**, Groop L, Salehi A. J Diabetes Complications. 2018 Sep; 32(9):813-818.

Abbreviations

AC	Adenylate cyclase
ADCYAP1	Adenylate cyclase activating polypeptide 1
AD	Alzheimer's disease
ADA	American Diabetes Association
Adgrg1	G protein–coupled receptor G1
ADGRG1/GPR56	Adhesion G-protein coupled receptor G1
aGPCR	Adhesion GPCR
AKOS	AKOS022075291
AMPK	AMP- activated protein kinase
ANT	Adenine nucleotide translocase
Aβ	Amyloid beta
BIBO	BIBO 3304 trifluoroacetate
Bt_2-cAMP	Non-metabolized cAMP analogue
BVDF	Polyvinylidene difluoride membrane
C	Membrane capacitance
cAMP	Cyclic adenosine monophosphate
cDNA	Complement DNA
ChREBP	Carbohydrate response element-binding protein
Coll III	Collagen III
C1QL1	Complement component 1, q subcomponent-like 1
CREP	Cyclic AMP responsive element binding protein
Cyp-D	Cyclophilin D
DIO	Diet induced obesity

DM	Diabetes Mellitus
DPP4	Dipeptidyl peptidase 4
Ep300	Histone acetyltrasferase p300
ER	Endoplasmic reticulum
GDM	Gestational diabetes mellitus
GDP	Guanosine diphosphate
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide
GPCRs	G protein-coupled receptors
Gpr142	G protein–coupled receptor 142
GSIS	Glucose-stimulated insulin secretion
HbA1c	Glycated hemoglobin
HD	Huntington's disease
HK	Hexokinases enzymes
HOMA-IR	Homeostasis model assessment
HRP	Horseradish peroxidase
IAPP	Islet amyloid polypeptide
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-β	Interleukin-1β
IMM	Mitochondrial inner membrane
iNOS	NOS2
IP3	Inositol trisphosphate
IPGTT	Intraperitoneal glucose tolerance test
K^+_{ATP}	ATP-sensitive K^+

KD	Knock-down
MCU	Mitochondrial Ca^{2+}
MGB	Minor groove binder
MODY	Maturity-onset diabetes of the young
mtCK	Mitochondrial creatine kinase
NFQ	Non-fluorescent quencher
NMRI	Naval Medical Research Institute
OCR	Oxygen consumption rate
OGTT	Oral glucose tolerance test
OMM	Mitochondrial outer membrane
PD	Parkinson's disease
PDE	Phosphodiesterase
Pdyn	Prodynorphin
PIP2	Phosphatidylinositol Biphosphate
PKA	Protein kinase A
PKC	Protein kinase C
plVDAC1	Plasma membrane-targeted protein
PPARγ	Peroxisome proliferator activated receptor
Ppy	Pancreatic polypeptide
PTP	Permeability transition pores
Pyy	Peptide YY
ROS	Reactive oxygen species
Rspo4	R-spondin-4
RT-qPCR	Quantitative polymerase real time PCR
SGLT2	Sodium-glucose co-transporter type 2

siRNA	Small interfering RNA
SNPs	Single-nucleotide polymorphisms
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TCA	Tricarboxylic acid
UCP-2	Uncoupling protein-2
VDAC1	Voltage-dependent anion channel-1
WHO	World Health Organization

Introduction

Diabetes Mellitus

Diabetes Mellitus (DM) is a heterogeneous metabolic disorder that is caused by multiple aetiologies. DM is characterized by chronic hyperglycaemia as a consequence of defective insulin secretion or insulin action or both [1, 2]. The development of diabetes is the last stage succeeding several pathogenic defects occurring in the pancreatic β-cells resulting in increased blood glucose [3]. The recognition of diabetes as a disease started in the 17th and 18th century in Europe, with describing patient's symptoms and distinguishing the linked obesity, which was noted in some cases, but it was not until the 20th century when the full description of diabetes was set in concomitant with the discovery of insulin [4]. Globally, there is a huge increase in incidence of the disease during the past 40 years, being around 108 million in 1980 to around 422 in 2014, making DM the 7th leading cause of death by 2030 according to the World Health Organization (WHO) [5].

Diabetes is a disease of both developing and developed countries, which puts a heavy burden on society and healthcare because of its severe complications. These complications are either microvascular, including retinopathy, nephropathy and neuropathy or macrovascular complications involving atherosclerosis in the coronary and cerebral vessels [6]. Developing a new effective therapy is not possible without fully understanding the disease pathophysiology. That is why great efforts have been paid worldwide to understand the causative factors behind this metabolic disease in order to find new drug therapies and improve the readily available ones. All these efforts aim at improving life quality for diabetic patients and prevent or minimize the disease-related complications [7].

Several factors are involved in maintaining normal fasting blood glucose levels within very narrow range *i.e.* between 3.5-5.5 mM, mainly through controlling glucose production and metabolism [8]. In these processes, several key hormones are involved, which are secreted from the pancreas, adrenal gland or the gastrointestinal tract such as insulin, glucagon, epinephrine, norepinephrine, cortisol, and incretin hormones. New insight has led to study the pathophysiological changes distressing the pancreatic islets of Langerhans and its association with the

disease initiation and progression, therefore DM is classified according to onset, pathophysiology and prognosis to:

Type 1 diabetes (T1D): that reportedly is caused by very rapidly decreased capability of producing insulin due to the destruction of β-cells, either by an autoimmune process or yet to be identified factors in genetically susceptible individuals. The resulting insulin deficiency usually starts in childhood or early adolescence and requires immediate insulin treatment and continuous monitoring for life [6, 9].

Type 2 diabetes (T2D): is a chronic disease caused by the interaction of genetic, epigenetic, environmental, and lifestyle factors. The hallmarks of T2D are chronic hyperglycaemia, insulin resistance or reduced insulin sensitivity. It is a multi-organ disease, including the pancreatic islets, the liver, adipose tissue, muscle, gut, and brain [1, 4].

Monogenic diabetes syndrome: this syndrome include; Maturity-onset diabetes of the young (MODY), an autosomal dominantly inherited early-onset disease [10]. And Neonatal Diabetes Mellitus (NDM), onset in the first 6 months of life, with an autosomal dominant or recessive inheritance [11]. Both are characterized by persistent hyperglycaemia due to partial or complete insulin deficiency.

Gestational diabetes mellitus (GDM): is caused by reduced insulin sensitivity in the peripheral tissues, mostly due to the elevated pregnancy hormones resulting in glucose intolerance. GDM mostly appears in the 2^{nd} or 3^{rd} trimester without previous encounter of DM [12].

Secondary DM results from diseases affecting the pancreas, such as pancreatitis, hormone disturbances as in Cushing's disease, or drug treatment, including corticosteroids. Secondary DM in some cases may be reversed by treating the primary cause [13].

Clinically, patients with severe DM present with polydipsia (thirst), polyphagia (hunger), polyuria (excess and frequent urination), unexplained weight loss, fatigue, blurred vision, numbness in the extremities and recurrent or severe infections [7]. Patients may spend years asymptomatic only to be discovered by routine examination (T2D) or strikingly presented with diabetic ketoacidosis (mostly T1D), which is a state of severe insulin depletion causing significant symptoms that require acute medical interventions [14].

According to the American Diabetes Association (ADA), the diagnosis of DM is based on both; clinical manifestations and plasma glucose levels using one of the following laboratory investigations [15]:

1- Fasting blood glucose above 7 mM (126 mg/dl).

2- Oral glucose tolerance test (OGTT), glucose levels are equal or above 11.1 mM (200 mg/dl) at 2 hours after 75g glucose load.

3- Glycated haemoglobin (HbA1c), an indicative marker for the average glucose levels during the last 3 months. HbA1c above 6.5% (48mmol/mol) is considered a criterion for DM.

The course of T2D

T2D is a heterogeneous multifactorial disease that involves carbohydrates, lipids and protein metabolism with deficient action of insulin on target tissues. The gradual development of insulin insensitivity in the peripheral tissues makes insulin action inadequate [16]. Genetically predisposed individuals are at higher risk of developing T2D when exposed to harmful environmental factors. More than 90 risk genes based on single-nucleotide polymorphisms (SNPs) were identified in individuals at higher risk of developing T2D, marking them susceptible for developing T2D [17]. Examples of genes that impose an increased risk of T2D are *CAPN10*, *TCF7L2, CDKAL1* and *KCNJ11*. Each gene affects different pathways resulting in increased individual disease susceptibility [18, 19]. For example, the *TCF7L2* transcription factor, which is part of the Wnt signalling pathway, has the strongest association with T2D. This candidate gene is involved in β-cell growth and survival as well as its regulation of glucose-stimulated insulin secretion (GSIS) [20]

Patients may remain asymptomatic for years, in what is conveniently termed the prediabetes state, during which they start to develop one of the following; impaired fasting glucose (IFG), when fasting blood glucose is between 5.6-6.9 mM and/or impaired glucose tolerance (IGT) when glucose levels at 2 hours during an OGTT lies between 7.8-11 mM and/or HbA1c is between 5.7-6.4% (39-47 mmol/mol) [15]. Prediabetes is associated with abdominal or visceral obesity, dyslipidaemia with high triglycerides and/or low HDL cholesterol, and hypertension [21].

A series of pathophysiological changes lead to the transformation of a prediabetic state to a clear-cut T2D. The process starts with defective insulin secretion in combination with insulin resistance in the peripheral tissues, caused by bad dietary habits, obesity, elevated plasma free fatty acids and reduced physical activity [22]. The presence of insulin resistance leads to a gradual increase in fasting plasma glucose up to 5.6 mM and disrupts the first phase of the normal biphasic insulin secretion despite an increase in the second phase secretion [23]. Over time, first phase insulin secretion in response to nutrients becomes blunted and patients with

mild or moderate hyperglycaemia develop severe postprandial hyperglycaemia [24]. Combined with the loss of first phase insulin secretion, hepatic glucose production increases, but due to insulin resistance, this glucose is not cleared from the circulation by uptake in the peripheral organs [25]. With an increase of fasting plasma glucose up to 7.8 mM, a further decrease in glucose clearance occurs till reaching a plateau around 7.8-11 mM accompanied by a decline in the compensatory insulin hypersecretion from the β-cells, which accelerates the development of overt diabetes [26]. However, the molecular assaults occurring in the β-cell during these pathophysiological changes need to be thoroughly investigated.

The pancreas and Islets of Langerhans:

The pancreas is one of the largest glands in the human body. It is composed of two different types of glandular tissue; the exocrine pancreas, which is a lobulated and acinar gland that synthesizes and stores digestive enzymes. These enzymes are secreted into ducts that gradually increase in size and terminate in the duodenum through the main pancreatic duct [27]. The other secretory part is the endocrine pancreas composed of the Islets of Langerhans, first described by Paul Langerhans in 1869, and they are scattered through the exocrine tissues. The secretory products are emptied into capillaries that connect the islets with the systemic circulation [28].

These spherical multicellular clusters of cells represent about 2-3% of the total pancreatic tissue in a healthy individual. The islets are supplied by a complex blood supply and innervation [29, 30] and are composed of different hormone secreting cell types: 20-30% α-cells (secreting glucagon), 60-80% β-cells (secreting insulin), 5-10% δ-cells (secreting somatostatin), γ-cells (secreting pancreatic polypeptide) and about less than 1% ε-cells (secreting ghrelin). Human islets have relatively more α-cells and fewer β-cells than rodents, and also cells are heterogeneously distributed throughout the islet although in some islets the β-cells are centrally located, whilst in rodents; β-cells are more distinguished in the central core of the islets surrounded by α-cells and δ- cells [31] (Figure1).

Cells within an islet interact and influence each other through autocrine and paracrine effects, in addition to gap junctions, permitting the harmony of action between the secreted hormones [32]. The β-cells play an essential role in the regulation of metabolism and energy homeostasis by secreting insulin and stimulating the storage of glucose as glycogen in the liver and glucose uptake in peripheral tissues [33]. The generation of β-cells starts during the foetal development with a short period of proliferation that takes place mostly during the first two years after birth [34]. It is still controversial whether β-cells undergoes

neogenesis throughout life, as evidence suggests that β-cells replication in adult humans is very limited [35].

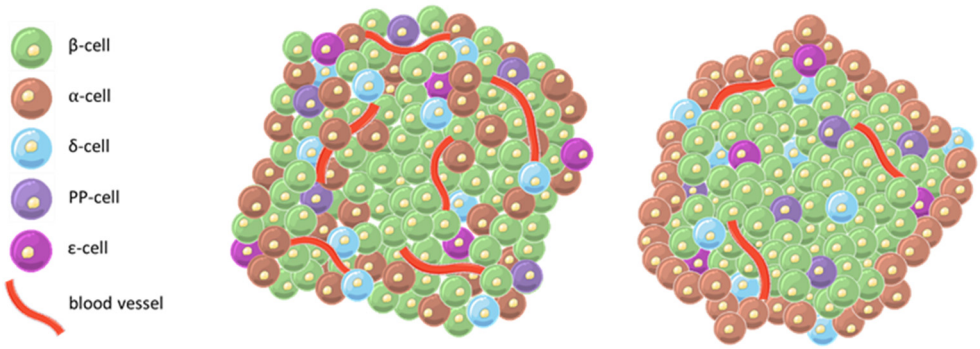

Figure1. Schematic cytoarchitecture of the islets of Langerhans in rodents (right) and humans (left) showing that cells in the human islets are heterogeneously distributed while in rodents, β-cells are centrally located surrounded by α-cells and δ- cells.

Insulin release from the β-cells

In β-cells, the insulin mRNA is translated as a single chain precursor (110 amino acids) termed preproinsulin. During processing in the endoplasmic reticulum (ER), the signal peptide at the N-terminus (24 residues) is removed to generate proinsulin (81 amino acids), which is folded before further transport to the next cellular compartment. In the Golgi apparatus, the proinsulin that consists of three domains: the β chain (amino-terminal), the α chain (carboxy-terminal) and a C peptide (connecting peptide in the middle) is exposed to further modifications in the secretory granules that excise the C peptide to produce the mature insulin in its crystalline form. Insulin is packaged into secretory granules, which are stored in the cytoplasm in preparation for secretion [36, 37].

Insulin granules are secreted from the β-cells in response to many stimuli; glucose, amino acids, fatty acids as well as neurotransmitters and hormones. To sense the circulating glucose, the β-cells are equipped with Glucose transporters (GLUT1, 2 and 3 in humans and GLUT2 in rodents), which facilitate diffusion of glucose into the β-cells, starting the stimulus- secretion coupling process (Figure 2). Glucose is then phosphorylated by the glucokinase enzyme to produce the metabolic substrate pyruvate via glycolysis [33]. In the mitochondria, pyruvate is oxidized through the tricarboxylic acid (TCA) cycle, yielding ATP by stimulation of the respiratory chain. The gradual build-up of ATP inside the β-cells causes an increase of the intracellular ATP/ADP ratio leading to the closure of the ATP-sensitive K^+ (K^+_{ATP})

channels. At rest, the plasma membrane sustains a negative resting potential with a high concentration of potassium intracellularly. Under glucose stimulation, the plasma membrane depolarizes, triggering voltage-dependent L-type Ca^{+2} channels opening for the influx and accumulation of Ca^{+2} resulting in activation of exocytosis of the docked insulin-containing granules [38].

Insulin is secreted in a biphasic manner in response to glucose. After a meal, insulin peaks rapidly within 5-7 minutes in what is termed the first phase insulin secretion, followed by a slow, yet sustained insulin release and this is the second phase of insulin secretion [39]. It is hypothesized that the first phase is prevailed by the release of insulin from the pre-docked granules, which are located immediately beneath the plasma membrane whilst the newly recruited granules by stimulation, which are first docked and then fused to the plasma membrane, are responsible for the second phase of insulin secretion [40].

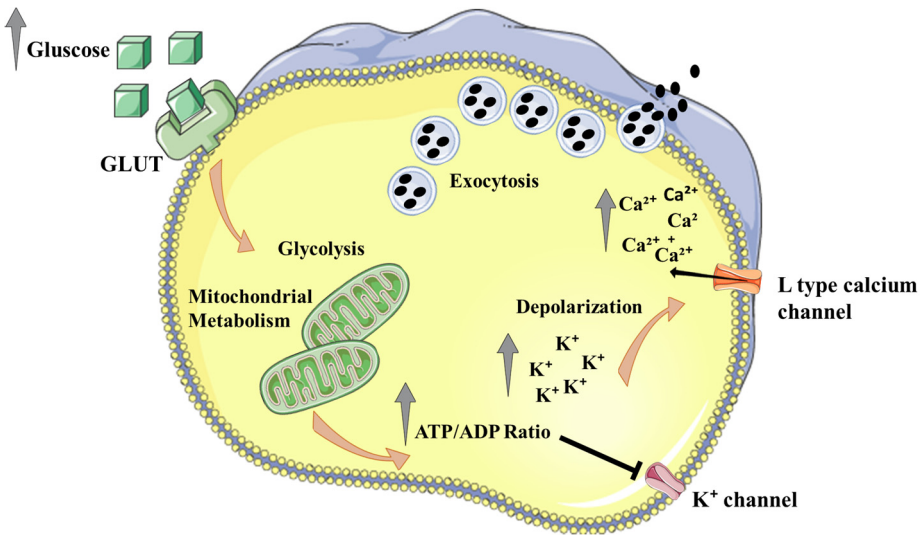

Figure 2. Schematic representation of stimulus-secretion coupling in the pancreatic β-cell starting by glucose sensing by the GLUT, increase in ATP/ADP ratio, closure of the K^{+}_{ATP} channels and plasma membrane depolarization opening the Ca^{+2} channels activating activation of exocytosis.

Role of cyclic adenosine monophosphate (cAMP) in insulin secretion

The second messenger cAMP is synthesized from ATP by the enzyme adenylate cyclase (AC) in the inner side of the plasma membrane and its concentration is balanced through degradation by cyclic nucleotide phosphodiesterase (PDE) [41]. Glucose sensing in the gastrointestinal tract results in the release of several peptides. These peptides, generally termed incretins, are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). They enhance the production of cAMP after binding to specific receptors on the β-cell surface [42, 43] (Figure 3). The function of cAMP is to: (i) transfer signals from the cell membrane to the intracellular compartments to cause an effect. (ii) Bind to and regulate the function of ion channels and (iii) activate protein kinases, which reversibly phosphorylate proteins and render them active or inactive [44, 45].

In the β-cell, cAMP potentiates glucose-stimulated insulin release by increasing Ca^{+2} influx through voltage-dependent Ca^{+2} channels and improves β-cell survival through protein kinase A dependant pathway (PKA) [46]. Additionally, an emerging evidence links cAMP- regulated guanine nucleotide exchange factor, (Epac) and control regulation of Ca^{+2} signalling and exocytosis in a PKA-independent pathway. By binding cAMP, Epac activates Rap1 and Rap2, two small GTPases of the Ras family, enhancing cell adhesion, gap junction formation and activation of phospholipase C enzyme (PLC) [47, 48].

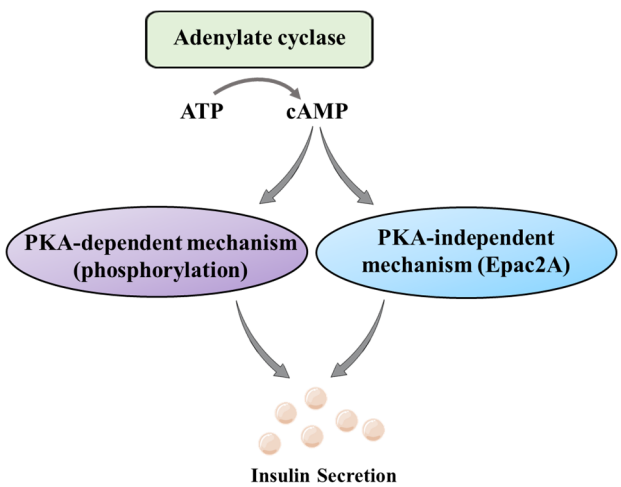

Figure 3. The generation of cAMP from ATP and its role in activation of two major pathways that play a major role in the stimulation of insulin secretion.

Causes of β-cell failure in T2D

Pancreatic β-cell dysfunction is the major cause for the pathogeneses of T2D [49]. Some reports also suggest that the damage process may even start in utero and early childhood, where it was proposed that malnutrition in utero, as well as exposure to hyperglycaemia, may precipitate to a higher risk of developing T2D in adulthood [50, 51]. The explanation is that these conditions impair β-cell development and prevent later adaptation to over nutrition. Nevertheless, this theory can only be included as one risk factor among many because not all individuals with early life malnutrition develop T2D in adulthood if they become obese [52].

Talchai *et al* 2012 studied mouse islets lacking the β-cell transcription factor Foxo1 and suggested β-cell dedifferentiation, a process which was identified in mice but not completely in humans [53]. The proposed β-cell dedifferentiation to a progenitor like state, is based on the fact that the loss of β-cells is associated with a concomitant increase of α-cells in these mice [54]. These dedifferentiated cells had lost their insulin expression as well as both β-cell transcription factors PDX1 and MafA [54]. In addition, they expressed high levels of the endocrine progenitor markers NGN3, OCT4, L-MYC and MANOG and started to express glucagon and the transcription factor MAFB, which is expressed in α-cells [55]. Although complete dedifferentiation of the cells was reported, it appears that some cells are still in the process. Brereton *et al* 2014 detected the presence of cells of β-cell origin that express neither insulin nor glucagon. Interestingly, other cells were found to be bi-hormonal (double positive), as they retained expression of PDX1 and GLUT2, and MAFB all together [56]. Similar to diabetic mice, 17% of islets from T2D donors have cells that cease to express any of the islet hormones (insulin, glucagon, somatostatin or pancreatic polypeptide), as compared to 6.5% in non-diabetic donors [57]. Along with that, co-expression of insulin and glucagon was found in about 1% of islets from 3 T2D donors reported by White *et al*, 2013 in concomitant with the expression of the mesenchymal marker vimentin, which indicates the plasticity of the adult β-cells [58]. Regardless of all these published data, the mechanism of dedifferentiation is not fully proved.

On the other hand, it was demonstrated without any controversy that prolonged glucotoxicity and lipotoxicity adversely affect β-cell function in complex and multifactorial mechanisms [59, 60], though it is still debated whether glucolipotoxicity causes morphological or functional changes in the β-cell. Morphological changes are represented by reduction in β-cell mass, varying from little to about 40-60%, seen in subjects with T2D as compared to non-diabetic control [61-63]. This reduction was found to be in both the total pancreatic weight and islet density. On the other hand, it was also verified that removal of 30-50% of the pancreas in humans has little effect on diabetes incidents, despite the fact that total insulin release was reduced by 50-90% [64-66]. Adding to that, complete

remission of diabetes was noticed in patients who had undergone bariatric surgery and within only few days, when the total weight loss is 1-2% [67, 68], which contradicts the reduction of the β-cell mass theory, since the β-cells have a very limited regeneration rate [69], thus making functional defect of the β-cell more likely as a cause of β-cell failure.

Impaired β-cell function can be related to oxidative stress, ER stress and mitochondrial dysfunction [70]. Under glucolipotoxic conditions, both PDX1 and MafA activities are reduced with subsequent inhibition of insulin gene expression. Additionally, the increased demand to produce more insulin triggers the unfolded protein response and sets ER under persistent stress and eventually leads to β-cell death by apoptosis [71, 72]. On top of that, the mitochondria are directly affected by hyperglycaemia, diminishing the ATP and disruption of Ca^{+2} homeostasis. This load on the mitochondria produces reactive oxygen species (ROS), as evidenced by increased markers of oxidative stress in T2D islets, which negatively impacts β-cell function [73, 74].

Another mechanism involves lipotoxicity in increasing uncoupling protein-2 (UCP-2) activity, a mitochondrial uncoupling protein that uncouples substrate oxidation from ATP synthesis, resulting in impairment of ATP generation from glucose metabolism and reducing GSIS [75]. Moreover, inflammatory products and toxins also negatively affect insulin secretion by enhancing β-cell production of the proinflammatory cytokine interleukin-1β (IL-β), the expression of which were found in the T2D pancreatic section and not in healthy subjects [76, 77]. At higher concentration, IL-β impairs insulin secretion and enhances apoptosis mediated by Fas, a member of the tumour necrosis receptor family, independent of TNF-α [78].

To summarize, the β-cell has an incredible ability to adapt and compensate for chronic hyperglycaemia, but overweight associated with long-standing insulin resistance heavily burdens the β-cell to produce more insulin until complete exhaustion. The generation of reactive oxygen species, induction of pro-apoptotic signals and ER stress are evident in β-cells from T2D donors as a proof of this exhaustion. Finally, it is important to understand the mechanisms, caused by excess glucose and lipids that lead to β-cell dysfunction and failure particularly in late stages of diabetes for correctly addressing the best mechanism to maintain the β-cell functioning for a longer period in T2D.

G protein-coupled receptors (GPCRs) in the β-cells

GPCRs are considered one of the largest class of cell surface receptors containing several hundred members and representing about 4% of the human genome [79]. They are composed of seven transmembrane domains anchored in the cell membrane and function to enable their ligands to cause effects inside cells [80]. GPCRs are classified into five major families; the largest is the rhopsin–like family constituting about 90% of the GPCRs and encoded by 273 genes. The remaining four families include secretin-like encoded by 48 genes, glutamate encoded by 22 genes, adhesion receptors and frizzled/taste 2 receptors encoded by 11 genes [81]. Each receptor has an amino-terminal domain, which enables the receptor to interact with the extracellular matrix, binding to its ligand and also has a carboxylic acid terminus that interacts with the intracellular cytoplasmic proteins. The amino-terminal domain differs in length and conformation among groups allowing them to discriminate between their ligands. Amisten *et al* reported the detection and quantification of mRNAs encoding 293 functional GPCRs in human islets that represents 76% of all known functional GPCRs [82].

The non-activated receptor is bound to G-protein in its Guanosine diphosphate (GDP)-bound state. G-proteins are complex trimeric proteins, composed of three subunits; α, β and γ located inside the cell (Figure 4). Upon receptor activation by agonist binding, GDP is replaced by Guanosine triphosphate (GTP). This exchange of molecular bindings allows the receptor to dissociate from G-protein and triggers the separation of α, β and γ subunits which intern starts a cascade of intracellular signalling pathways, most importantly, activation of the AC and the PLC enzymes [83, 84]. Both pathways, termed Gαs and Gαq respectively, are considered to have a stimulatory effect on insulin secretion.

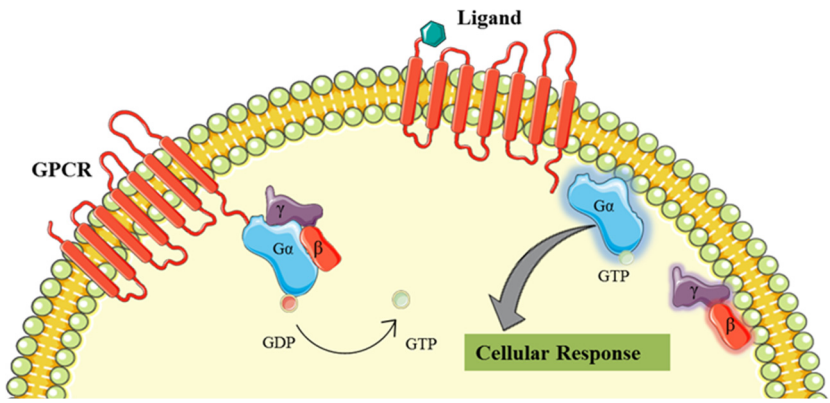

Figure 4. Schematic representation of GPCR activation upon binding with its ligand. The activated receptor dissociates from the G-protein complex initiating the cellular response.

Activation of AC produces cAMP and the latter binds to PKA and EPAC to stimulate insulin secretion. Another function of cAMP is to phosphorylate cyclic AMP responsive element binding protein (CREP). With CREP activation in the nucleus, the transcription of other genes is initiated [85]. The activation of PLC hydrolyzes phosphatidylinositol-bisphosphate and results in the generation of inositol trisphosphate (IP3). IP3 carries the signal to the endoplasmic reticulum to open the IP3 receptor Ca^{2+} ion channel, resulting in an increase in cytosolic Ca^{2+}. Diacyl-glycerol (DAG) is simultaneously generated by PLC, causing activation of protein kinase C (PKC) to enhance the granules translocation and start the secretion process [86]. GPCRs can have an inhibitory effect as well, which is represented, among others, by reduction of AC enzymatic action via the inhibitory GTP-binding protein Gαi [82] (Figure 5). Together, stimulatory and inhibitory effects control the β-cell function, secretion and viability.

The fact that GPCRs are targeted by about 70% of the current drugs, particularly for those receptors that bind peptides, makes GPCRs a great pharmaceutical interest. However, most of those receptors are still orphan, without any known ligands, which require extensive research to explore their therapeutic potentials [87]. We were interested in investigating two newly deorphanised receptors; ADGRG1/GPR56 and GPR142 and study their function and effect on β-cells and insulin secretion.

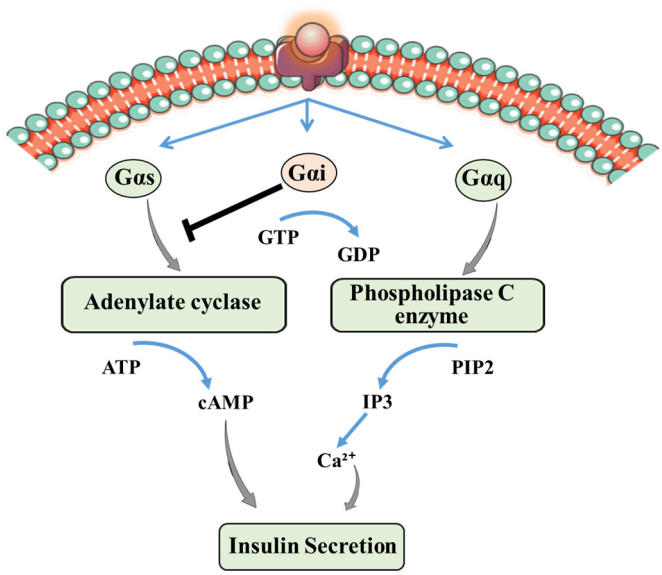

Figure 5. Activation of GPCRs and subsequent effect inside the β-cells. Gαs pathway enhances the production of cAMP, whilst Gαq pathway results in the generation of inositol trisphosphate (IP3) from Phosphatidylinositol Biphosphate (PIP2), which in turn increases cytosolic Ca^{2+} and augments insulin secretion

ADGRG1/GPR56

ADGRG1 formerly called GPR56 belongs to the adhesion GPCRs (aGPCR) family, is widely expressed in the central nervous system in both man and rodents [88]. ADGRG1 is characterized by an extremely long N-terminal extracellular domain, which is cleaved off from the membrane spanning C-terminal domain via auto-proteolysis [89]. A previous study has shown that ADGRG1 is the most abundant GPCR in rodents and human islets [82]. By interacting with the G-protein Rho, a member of the Ras superfamily of small GTPases via its effector ROCK, Rho exerts modulatory effects on the cell function [90]. Structurally, these aGPCRs attach to the extracellular matrix by an extremely long amino terminus, through which they attach to collagen type III (Coll III), their recently identified ligand initiating signalling via RhoA [91]. Coll III is usually found in the pancreatic extracellular matrix of both rodents and humans and functions to assist cell–cell and cell–matrix interactions [92]. Similarly, Olaniru *et al* demonstrated that GPR56 is highly expressed in rodents and human islets and exclusively within > 95% of the β-cell [93].

ADGRG1/GPR56 has been found highly expressed in the CNS of both man and rodents and a necessity for the normal brain function [94]. Li *et al* showed that Gpr56 knockout mice develop neuronal ectopias and regional lamination defects resulting in cortical dysplasia, whilst no cortical defects were observed in heterozygous animals [94]. Furthermore, mutations in the gene were linked with severe developmental brain disease and both overexpression and down regulation are associated with human gliomas [95]. This could be due to complete abolishment of binding ability between the ligand and the receptor in disease-associated mutations of the CNS [96]. Although ADGRG1/GPR56 is highly expressed in pancreatic β-cells, its correlation with β-cell dysfunction and T2D is not fully investigated.

GPR142

GPR142 is a novel, recently de-orphaned, receptor that belongs to the GPCRs family (rhopsin family). It is expressed in several tissues, with the highest expression being in the stomach, the duodenum, and the pancreas [97]. It was found that the expression of GPR142 is confined to the pancreatic islets rather than the whole pancreas, suggesting an endocrine specificity [97]. Interestingly, both α and β-cells express GPR142. GPR142 senses calcium and recognizes aromatic amino acids such as phenylalanine and tryptophan as its agonists [98].

Hua *et al* showed that activation of GPR142 by tryptophan in α-cells results in increased GLP-1 production and secretion independent of ambient glucose while intracellular glucagon content was not elevated [99]. Yoko *et al* studied GPR142 expression in *ob/ob* and diet-induced obesity (DIO) mice and found that in the fasting state, receptor expression was unaffected, contrary to a marked increase of expression seen after a meal. These mice were also reported to significantly improve fasting blood glucose and serum insulin levels after ingestion of tryptophan in comparison to the control mice fed with standard diet, suggesting enhanced responses of pancreatic beta cells [100]. Whilst the function of GPR142 has been studied in gastric ghrelin cells and intestinal K-cells [101, 102], their role in regulating insulin secretion and controlling glucose metabolism needs further intensive research. Previous screening studies performed in Hela cells overexpressing Gpr142 have suggested GPR142 being a Gαq-coupled receptor [97, 103], but in our research, we report GPR142 as Gs-coupled receptor augmenting insulin secretion and improving glucose tolerance.

The mitochondrial voltage dependent anion channels (VDAC)

VDAC, also known as mitochondrial porins, are located in the mitochondrial outer membrane (OMM) and function as a gatekeeper to cross-talk between mitochondria and cytoplasm by transporting small ions (e.g. phosphate, ATP and ADP), cations (e.g. Ca^{2+}, Na^{+} and K^{+}) and metabolites [104, 105]. VDAC has three distinct isoforms; VDAC1, VDAC2 and VDAC3, and accounts for approximately 0.4 % of the total mitochondrial protein and 50% of the total outer mitochondria membrane protein [106]. In most cell types, VDAC1 is the most abundant form, as shown by real time PCR and immunological as well as proteomics methods [107]. VDAC1 and VDAC2 are practically ubiquitously expressed, including pancreas as well as brain, heart, liver, skeletal muscle and kidney. VDAC3 is the least studied of the isoforms. However, in the testis and spermatozoa [108], VDAC3 knock out mice have altered mitochondria with defective sperm structure and reduced motility, rendering these mice infertile [109].

VDAC protein ranges between 30-35 kDa and is formed by 19 β-strands, conferring a special barrel shape forming a pore with an N-terminal α-helical segment located close to the pore. The interior parts of the β-strands consist of hydrophilic residues, whereas the exterior part, which is exposed to the lipid environment, is hydrophobic [110, 111]. The flux of ions and metabolites through the VDAC channel is controlled by changing the channel selectivity and permeability. At low voltages (10 mV), the VDAC channel is stably open for up to 2h, whilst, at high positive or

negative potentials (>40mV) VDAC shows multiple sub-states with different ionic selectivity and permeability. When the trans-membrane voltage exceeds 20-30 mV, VDAC channel shifts to a closed state [112].

VDAC1 might also be expressed in the plasma membrane, which was proved by antibodies against the VDAC1 protein. Its oligomerization creates large pores in the cell surface, leading to lethal changes [113, 114]. The mechanism by which VDAC1 is transported to the cell surface is still unclear, but several mechanisms have been proposed. One mechanism suggested the involvement of alternative splicing, yielding the addition of a pre-sequence to the VDAC1 coding sequence. Another mechanism proposed a change in the VDAC1 polypeptide conformation, meaning that with an increase in the VDAC1 concentration, VDAC equilibrium is shifted from monomeric to oligomeric species [115] (Figure 6).

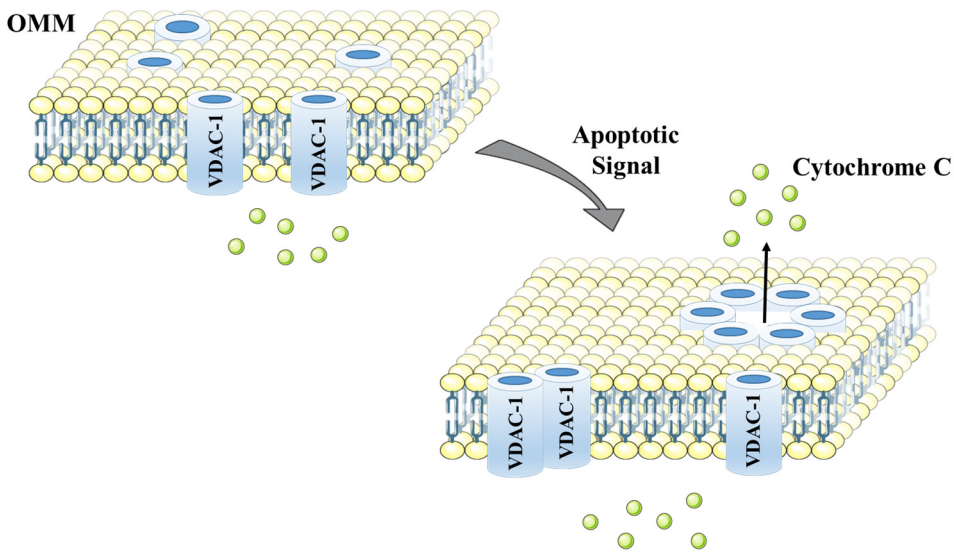

Figure 6. Schematic representation of the changes in VDAC1 conformation within the outer mitochondrial membrane resulting in subsequent creation of large pores that release cytochrome-c subjecting the cell to lethal changes.

This change in VDAC1 conformation also results in the creation of large pores in the mitochondria that release cytochrome-c and other proapoptotic proteins [116, 117]. Although normally interacting with the adenine nucleotide translocase (ANT) for normal ADP/ATP transport, it seems that as VDAC1 is accumulating, it forms a multiprotein complex with cyclophilin-D and the ANT. This leads to the leakiness of the tight mitochondrial inner membrane (IMM), mediated by mitochondrial

Ca^{2+}overload, forming and activating the permeability transition pore (PTP), with subsequent generation of reactive oxygen species [118]. Furthermore, it has been shown that VDAC is a hub protein, interacting with a large number of proteins, including both anti-apoptotic and pro-apoptotic members of the Bcl-2 family. The pro-apoptotic (Bax and tBid), modify the mitochondrial structure allowing cytochrome-c to exit through the OMM [119] (Figure 7). The role of VDAC1 in apoptosis was further proved in several studies by employing VDAC1 silencing, which revealed a reduction in cell death [120]. In contrast to VDAC1, VDAC2 was found to have an anti-apoptotic activity by inhibiting Bak-mediated activity [121].

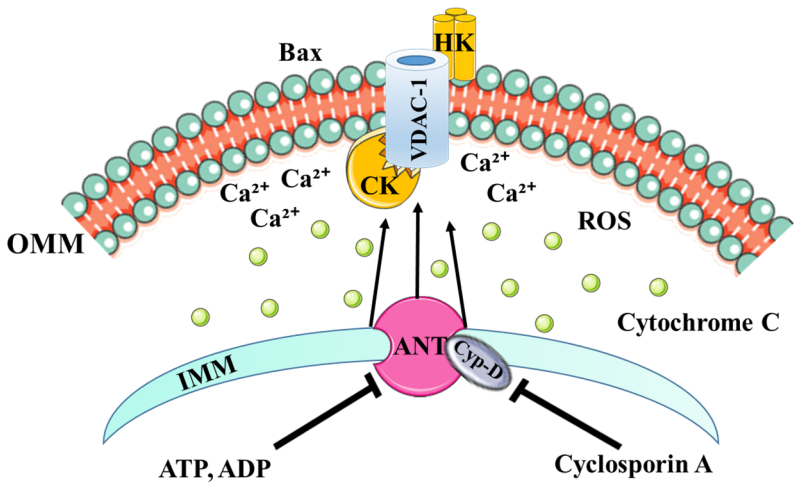

Figure 7. Activation of the permeability transition pores (PTP) within the Inner mitochondrial membrane and initiation of the apoptotic signal. PTT is composed of several trans-membrane proteins: the adenine nucleotide translocator (ANT), VDAC and the peptidyl- prolyl isomerase cyclophilin D (Cyp-D), In addition to associated proteins, such as hexokinase enzymes (HK) and mitochondrial creatine kinase (mtCK). It also involves members of the Bax/Bcl-2 family. VDAC conductance is indeed very different depending on its open state. Normally, it conducts Ca^{2+} when it is almost entirely closed, while negatively charged nucleotides such as ADP and ATP are conducted at more open states of the channel. The opening of the mitochondrial PTP results in Ca^{2+} overload and followed by mitochondrial swelling and OMM rupture.

Furthermore, VDAC1 transcript levels were found to be higher in human cancer cell lines as compared to normal fibroblast cells [122]. It seems that cancerous cell survival is dependent on VDAC1 overexpression, contributing to their metabolism, due to the binding of hexokinases (HK). Several studies reveal that VDCA1 down regulation interferes with VDAC1 interaction with HK resulting in inhibition of cancer cell and tumour growth [123, 124].

In addition to modulating apoptotic and tumorigenic activity, VDAC1 mediates transfer of Ca^{2+} between the ER and mitochondria through the OMM [125]. Several proteins are then responsible for transporting Ca^{2+} into the mitochondrial matrix, including the mitochondrial Ca^{2+}uniporter (MCU) and the Na^{+}/Ca^{2+}exchanger [126]. The presence of intramitochondrial Ca^{2+} is crucial for the normal function of critical enzymes in the TCA cycle, fatty acid oxidation, and amino acid catabolism and any disruption of the Ca^{2+} homeostasis can lead to overload and cause toxic effects on the mitochondria [127]. Previous studies in HeLa cells and skeletal myotubes showed that VDAC1 over-expression increases mitochondrial Ca^{2+} concentration, whilst using siRNA to silence VDAC1 results in reduced mitochondrial Ca^{2+} uptake and cell apoptosis as induced by H_2O_2 or ceramide [128]. These results apply to cells in which VDAC1 is expressed at much higher levels than VDAC2 [119].

Moreover, disruption of Ca^{2+} homeostasis in association with high-levels of VDAC1 had been long proposed as a cause for several neurodegenerative diseases such as: Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD) [129]. Post-mortem studies on brain sections from AD patients revealed dystrophic neurites (axonal or a dendritical projections from a neuron) of Amyloid beta (Aβ) deposits contrary to control subjects [130, 131]. Smilansky and colleagues demonstrated that Aβ interacts specifically with the VDAC1-N-terminal region and that the latter is mandatory for Aβ entry into the cell, mediating neuronal apoptosis [132].

VDAC1 gene deletion was reported in human patients, either as solitary deletion or as a consequence of other mitochondrial defects, but it is rare in occurrence, implementing a possibility of lethality in humans. However, VDAC1 KO-mice were viable with mitochondrial defects resulting in abnormal muscle physiology, contrary to VDAC2 deletion, which produced embryonically lethal offspring. Our previous studies showed an altered VDAC1/VDAC2 expression in insulinoma INS-1 cells under a glucotoxic condition although the detailed cellular mechanism was hitherto not investigated [133].

T2D drug treatment

Current treatment in T2D aims at controlling blood glucose and minimizing the complications (microvascular and macrovascular), but still, the average life expectancy for T2D patients is shortened by 5-17 years [134, 135]. The first advice given to the newly diagnosed T2D patients is to change their lifestyles by eating healthier and increasing physical activities, but these conservative measures sometimes are not sufficient to reach glycaemic control; therefore, drug treatment

must be added [136, 137]. Several drugs are available for treatment of T2D, with different mode of actions and side effects. Some of these drugs had been available for generations so far such as metformin and sulfonylureas, whereas some are new in the market e.g. the DPP-4 inhibitors, sodium-glucose co-transporter type 2 (SGLT2) inhibitors and 5 GLP-1 analogues. Drug treatment might be used alone or in conjugation with insulin therapy [138].

Metformin is the first line of treatment for T2D and prediabetes as it temporary prevent or delay the onset of the disease [139]. It acts on the liver to reduce gluconeogenesis rate and on the peripheral tissue to stimulate glucose uptake. The mechanism of action includes the activation of AMP- activated protein kinase (AMPK), which reduces gluconeogenesis gene expression [140]. The latter also interferes with acetyl CoA carboxylase cycle resulting in improvement of insulin action [141]. Another mechanism stated that metformin can directly inhibit gluconeogenesis by direct inhibition of enzyme fructose 1, 6-bisphosphatase [142]. Finally, metformin can mediate the effect of glucagon by direct inhibitory effect of AMP on adenylyl cyclase [143]. Generally, the gastrointestinal disorder following metformin treatment causes 15-20% of diabetic patients to refrain from taking metformin. There is evidence that metformin reduces the risk for myocardial infarction, a common complication for long term T2D [144], and has a protective effect against ocular complication such as cataracts, glaucoma, and diabetic retinopathy [145]. The only contraindication to metformin is impaired renal function in patients, for fear of lactic acidosis, a life-threatening condition characterized by low blood pH and elevated blood lactate levels [146, 147].

If metformin as a first line of treatment fails, ADA recommends adding one of the following second line treatments to metformin:

Sulfonylureas including glibenclamide, glipizide, gliclazide and glimepiride, are currently used to treat T2D patients. By binding to the Sulfonylurea receptor (SUR1) on the β-cell and closing the K^+_{ATP} channel, they succeed in increasing insulin secretion [148, 149]. Another important function of these drugs is their effectiveness in lowering plasma glucose levels and HbA1c; however weight gain and the risk of hypoglycaemic episodes are higher with this group of drugs [150]. One controversial aspect of this drug is that it has been suspected to cause β-cell exhaustion, as it seems that with longer treatment duration the drug causes a rapid decline in β-cell function resulting in deterioration of the glycaemic control [151].

Meglitinides have a similar mode of action to sulfonylureas by binding to receptor SUR1 on the β-cell and blocking the K^+_{ATP} channel with an advantage of not causing weight gain [152, 153]. They have a shorter plasma half-life with only 1-2 h as they tend to dissociate from the SUR1 receptor faster than Sulfonylureas [154].

This feature makes meglitinides suitable for use in patients with irregular meals and in the treatment of postprandial hyperglycaemia [155].

Thiazolidinediones is another drug class that functions to improve peripheral insulin resistance, by binding to and activating the nuclear receptor peroxisome proliferator activated receptor (PPARγ) [156]. Activation of this receptor induces transcription of genes with PPARγ binding sites, such genes are important for fat storage and adipocyte differentiation. The increase in adipogenesis contributes to weight gain in patients using this drug for a long duration, but fat is located mainly in subcutaneous tissue sparing fat uptake by the liver and muscle, hence promoting insulin sensitivity [157]. Although thiazolidinediones improve β-cell function over a short-term period, they have significant side effects other than weight gain, which include oedema caused by fluid retention which may lead to congestive heart failure [158, 159]. In addition, there is an increased risk of bone fractures with a higher risk in postmenopausal women. This could be due to bone marrow differentiation to marrow adipocytes in favour of osteoblasts leading to bone loss [160].

GLP1 receptor agonists and dipeptidyl peptidase 4 (DPP4) inhibitors, the GLP-1 hormone is secreted by intestinal L-cells in response to nutrients and is rapidly degraded (1-2 minutes) by the DPP4 enzyme [161]. GLP1 binds to Gs protein coupled receptors stimulating cAMP production resulting in enhancement of GSIS [162]. The currently available drugs that aim at increasing incretin receptor signalling are either GLP1 receptor agonists or DPP4 inhibitors. These drugs improve glycaemic control by increasing GSIS, slowing gastric emptying and increasing satiety with minimal risk of hypoglycaemia and modest weight loss [163, 164]. The only drawback is that GLP1 receptor agonists are administered by injection rather than oral tablets as in DPP4 inhibitors.

SGLT2 inhibitors are the newest drugs in T2D treatment that act on the kidney instead of the pancreas. SGLT2 prevents reabsorption of glucose from the proximal tubules resulting in a reduction of plasma glucose independent of insulin [165]. At the same time, they can improve peripheral glucose sensitivity with the advantage of significant weight loss and lowering blood pressure which makes it suitable for T2D patients with hypertension [166]. On the other hand, SGLT2 inhibitors introduce side effects related to glycosuria such as genital and urinary tract infections, which may lead to acute kidney failures in severe cases [167]. Besides, they have been shown to induce a slight increase in HDL cholesterol and glucagon levels in patients with risk of ketoacidosis [168].

However, it should also be mentioned that none of the existing T2D drugs mentioned above could preserve β-cell function and prevent β-cell death, which, could explain why insulin treatment for T2D becomes unavoidable.

Aims

Paper I- In this study, we wished to identify mRNA expression of all peptidergic ligands of islet GPCRs to generate "signalome" atlases mapping the pathways by which the peptide-ligands regulate human and mouse islet GPCR signalling. By doing this, we can also identify common or differentially expressed peptidergic ligands and their corresponding GPCRs in both human and mouse cells.

Paper II- Since GPR56/aADGRG1 is highly expressed in islets we wish to investigate the role of GPR56/aADGRG1 in pancreatic islet function and development of islet dysfunction in T2D. Moreover, we also wanted to study the correlation of GPR56/aADGRG1 transcript expression with other gene transcripts in human islets. The possible signalling cascade underlying GPR56/aADGRG1 activation was also studied.

Paper III- To examine and evaluate the possible role of VDAC1 and VDAC2 in pancreatic β-cell function we performed studies on isolated islets from diabetic and non-diabetic human cadaveric donors. We also investigated the mechanisms behind the synchronized changes in VDAC1 and VDAC2 under pathophysiological condition such as hyperglycaemia. The expression level of Vdac1 in pancreatic islet of an animal model of T2D *i.e. db/db* mouse was also was also desirable to investigate.

Paper IV- In the current investigation we wanted to further study whether Coll III has any impact on the major MAPk pathways or activation of transcriptional factors important for a proper β-cell function. Since Adgrg1-KD in β-cells was accompanied by a reduced viability and increased apoptosis, we wished to study the possible involvement of Vdac1 in this context.

Paper V- In this paper we wanted to investigate the functional impact of the previously deorphanized Gpr142 on cAMP generation in the rodent β-cell. Attempts were also implemented to study the effect of Gpr142 activation by selective agonists on the expression of VDAC1 as well as Chrebp and Txnip during glucotoxic condition.

Materials and methods

Human pancreatic islets

Isolated human pancreatic islets from non-diabetic, both males and females (HbA1c: 5.7±0.14) and T2D, both males and females (HbA1c: 6.4±0.25) were provided by the Nordic Network for Clinical Islet Transplantation (Olle Korsgren, Uppsala University, Sweden). Prior to the experiments, the human islets with the 70-90 % purity had been cultured at 37°C (5%Co_2) for 1-5 days in CMRL 1066 supplemented with 10 mmol/ 1 HEPES, 2 mmol/1 L-glutamine, 50 µg/ml gentamicin, 0.25 µg /ml fungizone, 20 µg /ml ciprofloxacin ,10 mmol/1 nicotinamide and 5.6 mM glucose. The islets were then hand-picked under stereomicroscope prior to use. Work on Human pancreatic islets was approved by the ethical committees at the Unversity of Uppsala and Lund, Sweden.

Animals

We used female mice of the Naval Medical Research Institute (NMRl) strain (B&K, Sollentuna, Sweden), weighing 25-30g. Inbred male mice of the c57Bl/6 (C57; Charles River) strain and outbred Crl:CD1 (ICR) aged 10-12 weeks. Also, female mice of the c57/Bl strain (Janvier Labs, Paris), weighing 25-30 g.

A good model of obesity and diabetes used was the *db/db* mouse ($Lepr^{db/db}$). These mice present with hyperphagia, obesity and insulin resistant caused by a mutation in the leptin receptor gene, which introduces a new splice site and results in a dysfunctional receptor [169, 170]. They develop severe diabetes in a short duration due to β-cell dysfunction, therefore it was important to use young mice 5-6 weeks.

All animals were given a standard pellet diet (B&K, Sollentuna, Sweden) and tap water *ad libitum* throughout the experiments. All animals were housed in metabolic cages with constant temperature (22ºC) and 12 hours light/dark cycles. The experimental procedures were approved by the Ethical Committee for Animal Research at the University of Lund; Sweden and were in accordance with the international standard recommended by NIH.

Intraperitoneal glucose tolerance test (IPGTT)

Both *db/db* and c57/bl mice were treated with daily intraperitoneal injection with VIBT-4 25 mg/kg body weight for 5 weeks. On the day of the test, mice were fasted for 4 h and were injected with Glucose (dissolved in 0.9% NaCl and 2.0 g glucose/kg body weight). Serial blood sampling from vena saphena was performed thereafter at 0, 5, 15, 30 and 90 minutes. Plasma Insulin was analysed by ELISA and blood glucose by glucose oxidase method.

Isolation of mouse pancreatic islets

Preparation of pancreas

The distal end of the pancreatic duct was clamped and then retrograde injection with approximately 3-5 ml of ice-cold collagenase solution via the bile-pancreatic duct was performed is previously described [171]. Thereafter pancreas was dissected out and carefully separated from the surrounding tissue and then placed in tubes (50 ml) and subsequently in water bath (30 cycles/minute) at 37°C for 17 min [172].

Isolation of islets

Pancreatic islets were separated from acinar tissue by vigorous Shaking in ice-cold Hank's solution for several minutes. After sedimentation for about 10-15 min, the islets were handpicked under stereomicroscope at room temperature and then subjected to different experimental procedures.

Glucose- stimulated insulin secretion in human and mouse islets

Freshly isolated islets or cultured islets (cultured in RPMI media containing 5 or 20 mM glucose in incubator 37°C, 5% CO_2 for 72h, in the presence or absence test agents) were preincubated for 30 min at 37°C in Krebs Ringer bicarbonate buffer, pH 7.4, supplemented with N-2 hydroxyethylpiperazine-N'-2-ethanesulfonicacid (10 mM), 0.1% bovine serum albumin, and 1 mM glucose. Each incubation vial contained 12 islets in 1.0 ml KRB buffer so solution and treated with 95% O_2 and 5% CO_2 to obtain constant pH and oxygenation. After preincubation, the buffer was

changed. The islets were then incubated with different test agents in a medium containing either 1mM or 16.7 mM glucose for 1h at 37°C in a metabolic shaker (30 cycles per min). An aliquot of the medium was removed immediately after incubation for the analysis of insulin and the islets were incubated in acid-ethanol for insulin content determination by Radioimmunoassay.

Cell Lines

INS-1 832/13 cells are a good model for stimulus- secretion coupling. They are originated from rat INS-1 insulinoma cells but were stably transfected with a plasmid encoding human proinsulin gene, which made these cells more responsive to glucose and other secretagogues. INS-1 832/13 cells are easy to keep and expand in larger experiments as they have short doubling time, but they are not identical to primary cells genotypically or Phenotypically [173].

Histone acetyltransferase p300 (Ep300) Knock-out cells. Basically, they are INS-1 832/13 which were done-regulated in by CRISPR/Cas9. We used this cell line to study the impact of up-stream signalling important for VDAC1 expression. The p300 protein functions as a transcriptional coactivator as it binds to transcription factors to activate the transcription machinery [174].

Both cell lines were cultured in RPMl-1640 containing 11.1 mM D-glucose and supplemented with 10% foetal bovine serum, 100 µg/ml streptomycin, l0mM HEPES, 2mM glutamine, 1 mM sodium pyruvate, and 50 µM β-mercaptoethanol, at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. After the initial culture period, the culture medium was replaced with medium 5 or 20 mM glucose in the presence or absence of indicated agents and culture continued for three days.

Insulin secretion in cultured INS-1 832/13 cells

INS-1 832/13 cells were kept in HEPES balanced salt solution (HBSS;114mM NaCl; 4.7 mM KCl,1.2 mM KH_2PO_4; 1.16 mM $MgSO_4$; 20 mM HEPES; 2.5 mM $CaCl_2$; 25.5 mM $NaHCO_3$; 0.2% bsa; Ph 7.2) supplemented with 2.8 mM glucose for 2 h at 37ºC. Thereafter the cells were incubated in a fresh media with the desired glucose concentration and test agents for 1h. after incubation an aliquot of the media was removed to analyse insulin and the cells were saved for the analysis of insulin content.

Biochemical and radio immunological analysis

Glucose oxidase method

A colorimetric procedure utilizes the oxidation of β-D glucose to produce D-glucono-1, 5-lactone and hydrogen peroxidase. Peroxidase enzyme breaks the later to produce oxygen free radicals, which are accepted by pigments that changes colours depending on glucose concentration. The colour intensity can reveal glucose concentration by the aid of standards [175].

Determination of insulin

The concentrations of insulin (total insulin and plasma insulin) was determined by RIA and ELISA radioimmunoassay kits. RIA is a competitive assay where insulin in a sample competes with radioactive ^{125}I-labelled insulin at a specific site on insulin-specific antibody [176, 177]. ELISA is a plate-based antigen-antibody reaction technique. The desired antigen (hormone, protein, antibodies, peptides) is sandwiched between fixed antibody on the plate and the enzyme-labelled detection antibody [178].

Determination of protein

Protein was determined according to the bicinchoninic acid assay (BCA assay), a colorimetric assay that relies on two reactions. First, reduction of Cu^{2+} ions from the copper (II) sulphate to Cu^{+} by the peptide bonds in protein. Then, chelation of each Cu^{+} ion with two molecules of bicinchoninic acid to form green to purple-coloured complex, which can then be measured using colorimetric techniques [179, 180].

cAMP measurement

For the measurement of cAMP INS-1 832/13 cells or mouse islets (50 islets) were incubated for 60 min at 1 or 16.7 mmoI/1 glucose in the presence or absence of the test agent. Incubation buffer 1.0 ml of Krebs-Ringer bicarbonate (KRB)-buffer including 100μmol/L IBMX (100 μM) to prevent the hydrolysis of cAMP by the cellular phosphodiesterase. After the incubation, the cells or islets were washed with PBS and stored in Ripa buffer containing 100 mM HCl and 100 μM IBMX for 5 min and then frozen. At the day of analysis samples were sonicated on ice and cAMP was measured using a cAMP ELISA kit (Enzo Life Sciences), according to manufacturer's instructions. The protein concentration of the islet lysates were measured by BCA kit as described above.

Caspase-3 activity

Measurement of caspase-3 gives an indication of β-cell damage and death during subjection to different treatments or toxic conditions. Caspase-3 protein plays a crucial role in cellular apoptosis. This programmed cell death is activated by two mechanisms; either by the extrinsic pathway through T-lumphocyte sending a death signal, activating Caspase-8, which in turn activates Caspase-3. The other mechanism is the intrinsic (mitochondrial) pathway, where cytochrome-C is leaked from the mitochondria of the damaged cell and activates the caspase cascade [181, 182]. It had been linked to Alzheimer disease where there is severe neuronal degeneration [183].

The activity of caspase-3 was determined by monitoring the cleavage of a specific fluorogenic caspase-3 substrate. The cells lysate were allowed to react with the fluorogenic caspase-3 substrate in a 96-well plate (3 µl to each well) in a reaction buffer containing HEPES (20 mmol/1), glycerol (10 %) and DTT (2 mM). The mixtures were maintained at 37° C for 60 min (darkness) and subsequently analysed in a fluorometer equipped with an excitation wavelength of 390 nm and an emission wavelength of 460 nm. The results were correlated to the protein concentration of each well.

Transfection of cells, mouse and human islets

INSl 832/13 cells were seeded in six-well plates at a density of ~5 x 105 cells in culture media without antibiotics. Predesigned small interfering RNA (siRNA) is used for silencing the desired genes. siRNAs are small (20-25bp), double-stranded RNA molecules, which causes the destruction of mRNA and thus inhibit the expression of their target genes. Lipofectamine transfection reagent was used for transfecting oligonucleotides into INSl 832/13 cells. It facilitates the delivery of oligonucleotides to the negatively charged cell membrane by entrapping them in positively charged lysosomes. Twenty-four hours post-transfection, the medium was replaced with fresh medium containing antibiotics. Seventy-two hours post transfection, cells were lysed to extract total RNA or protein to measure the knockout efficacy or subjected to further experiments [184, 185].

Mouse and human islets are down-regulated by using a cocktail of different Lenti-virus delivered shRNAs targeting the desired gene. Control islets (scramble transfected with lentiviral particles without targeting any specific region) were used for comparison. The islets were cultured for 72h and then subjected to further experiments [186].

Cell viability (cellular reductive capacity and apoptosis

Cell viability was performed on INS-1 832/13 cells (subjected to 5, 7 or 20 mM glucose for 72h in the absence or presence of test agents or dispersed mouse islet cells (using Ca^{+2} free medium) after being subjected to different experimental conditions (cultured with or without a cocktail of pro-apoptotic cytokines for 24h in RPMI 1640 with 5 µmol/L glucose and 10% FBS supplemented with 5 µmol/L ATRA. Measurement of cell viability and detection of apoptosis was performed using the MTS reagent kit (Promega according to the manufacturer's instructions. Cell death measured using ELISA^{PLUS} kit (Roche Diagnostics, which quantifies the appearance of cytosolic nucleosomes.

Cell proliferation assay

INS-1 832/13 cells were seeded at 10 000 cells/well into 48-well plates in RPMI1640 medium (Life Technologies with GlutaMaxTM (Gibco, USA containing 11,1 mM glucose and supplemented with 15% fetal calf serum, 50 µg/L streptomycin (Gibco),75 mg/L penicillin sulphate (Gibco) and 5 µL/mL β-mercaptoethanol (Sigma. Thereafter the cells were washed and transfected with siRNA or lentiviral particles targeting the desired gene as described above. After transfection (36h and a recovery period (6h in normal RPMI1640 culture medium, the plates were cultured for additional 72h in normal RPMI1640 culture medium at 37°C with 5% ambient CO_2. The cells (in individual wells) were then harvested by trypsination and counted using a Bürcker chamber.

Detection of mRNA expression by qPCR

To validate and quantify gene expression, and to assess the quality of knocked down gene, we used reverse transcription quantitative polymerase real time PCR (RT-qPCR to amplify the gene of interest. It is a two-step process, where the first step is to produce complement DNA (cDNA from the extracted mRNA (cells, mouse or human islets, using a reverse transcriptase enzyme, primers and dNTPs. Concentration and purity of total RNA was measured with a NanoDrop ND-1000 spectrophotometer (A260/A280>1.9 and A260/A230>1.4. In the second step, cDNA is used as a template for the exponential amplification using real time PCR TaqMan assays (5'nuclease assay process with primers and probes specific for each gene of interest [187].

The TaqMan®MGB probes contains a reporter dye (FAM^{TM} dye) linked to the 5'end of the probe, a minor groove binder (MGB at the 3'end of the probe a probe and a non-fluorescent quencher (NFQ at the 3'end of the probe. In the intact probe, the reporter fluorescence is suppressed due to the proximity of the reporter dye to the

quencher dye. When DNA polymerase cleaves the probes, it separates the reporter dye from quencher dye resulting in increased fluorescence by the reporter [188]. The increase in fluoresce signal occurs only if the target sequence is complementary to the probe and is amplified during PCR therefore nonspecific amplification is not detected. The amplification is logarithmic and, meaning that, in each cycle in the reaction leads to a doubling of the number of the targeted sequence. The expression of the gene of interest is then normalized against an endogenous control (genes whose expression levels does not differ between the investigated samples) using the ΔΔCt method [189, 190].

Western blot analysis

This technique is used for the detection and quantification of a certain protein after various treatments [191]. Complete protease inhibitor cocktail is added to the lysate from cells, sonicated mouse or human islets sample before treating it with sodium dodecyl sulphate and heated up to near boiling for a few minutes. This step is crucial to unfold the protein and make it negatively charged by breaking the disulphide bonds from secondary tertiary structure rendering the protein linear in structure [192]. The protein is then loaded on a SDS-Polyacrylamide Gel, and gel electrophoresis is run to allow migration of the negatively charged proteins toward the positive anode, during which, they separate according to molecular weight [193].

The proteins bands on the gel are transferred (plotted) onto a polyvinylidene difluoride membrane (BVDF) either by wet or semi-dry transfer. The membrane is then blocked to avoid non-specific bindings before adding the primary antibody against the protein of interest and allowed to be incubated for 24h. Secondary horseradish peroxidase (HRP) linked antibody specifically against the primary antibody is allowed to incubate for 2 h. A light signal (428 nm) is emitted when HRP catalyzes the oxidation of luminal to 3-aminophalyte which can be enhanced (by certain chemicals) and read by chemiluminescence (ECL-READER) [194]. For quantification total protein or a housekeeping endogenous protein such as β-actin cyclophilin is used and the analysis of the result is done using the appropriate Bio-Rad software.

Immunostaining and confocal Imaging

Immunostaining is a technique based on antibody-antigen reaction to visualize proteins or peptides inside the cell (immunocytochemistry) or tissue (immunohistochemistry). The primary antibody is built against the protein of interest, the antigen. The secondary antibody is labelled with a fluorophore that enables visualization [195]. Different lasers excite fluorophores in the secondary

antibody to emit light at different wavelengths, which can be visualized by the laser scanning confocal microscope (Zeiss LSM-510) and analysed by the Zen software (Zeiss, Germany).

Isolated human or mouse islets, as well as INS-1 cells, were seeded on glass-bottom dishes cultured overnight. Cells were then washed twice and fixed with 3% paraformaldehyde for 10 min, followed by permeabilization with 0.1% Triton-X 100 for 15 min. The blocking solution contained 5% normal donkey serum in PBS and was used for 15 min. Primary antibodies against protein of interest were diluted in blocking buffer and incubated overnight at 4°C. Immunoreactivity was quantified using fluorescently labelled secondary antibodies (1:200). The ratio was calculated by the mean intensity of plasma membrane to mean intensity in the cytosol, according to the formula presented in the paper.

Immunohistochemistry was performed following standard protocol. The human pancreases ere paraffin embedded, cut into 10 um thick sections and rehydrated and then stained with primary antibodies targeting the desired antigen.

Single cell ATP/ADP ratio measurement

Measurement of cellular ATP is an important indicator of the beta cell function and regulation of insulin secretion as described above. For an accurate measurement of metabolic regulation of ATP levels (ATP: ADP ratio) in the in beta cells, we used PercevalHR technique, which is a genetically-encoded fluorescence biosensor that detects the changes in ATP: ADP ratio in live single cells [196, 197].

INS-1 cells were co-transfected with either wtVDACl or des-Cys (127/232) VDACl plasmid together with PercevalHR (1 μg/ml). Single live cell imaging was performed by confocal microscopy and applied to INS-1 cells. Both ADP and ATP can be detected in the single cell at fluorescence at ≈420 and ≈500nm respectively and the ratio of ATP: ADP in the cells can be measured by the emitted fluorescence [198]. Expression levels were determined by qPCR.

Oxygen consumption rate (OCR)

OCR was used to determine cellular respiration in low (2.8 mM) glucose and for 60 mm following the transition to high (16.7 mM) glucose, which identifies metabolic disturbances due to ATP changes during glucotoxic conditions [199, 200]. OCR was measured in INS-1 832/13 cells using the extracellular flux analyser XF24 (Seahorse Bioscience), as previously described in detail [201]. An assay medium composed of 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.16 mM $MgSO_4$, 20 Mm HEPES, 2.5 mM $CaCl_2$, 0.2% bovine serum albumin, pH 7.2, and supplemented with 2.8 mM glucose was used in the XF analysis. The cells were

seeded in an XF24 24-well cell culture microplate at 250,000 cells/well (0.32cm² growth area) in 500 µl of growth medium and incubated overnight at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Prior to assay, RPMI 1640 medium was removed id replaced by 750 µl of assay medium. The cells were preincubated under these conditions for 2 h at 37°C in air. The proportions of respiration driving ATP synthesis and proton leak were determined by the addition of oligomycin (4 µg/ml). After a further 30 min, 4 µM of dinitrophenol was added to determine maximal respiratory capacity. After a further 10 min, 1 µM rotenone was added to block transfer of electrons from complex I ubiquinone.

Conductance measurement by patch clamp recording

Patch-clamp is an important technique that can study of the electrical properties of cells and tissues, where it utilizes a tight seal between the pipette and the cell membrane (electrical resistance of >1GΩ gigaohm). To perform the current measurements, a small patch of membrane is sucked into the pipette to create an omega-shaped semi-vesicle. This configuration allows the measurements of membrane potential, currents passing through the membrane, exocytosis and endocytosis events [202]. Currents can be measured by holding the cell at a negative membrane potential (-70 mV) in the voltage-clamp mode where membrane potential is controlled by the amplifier resulting in perforation of the membrane and the ion-channel activity can be studied. Conductance was measured continuously in the same single cell before, during and after the acute addition of the respective compound. The membrane capacitance (C) is given by the equation:

$$C = (\varepsilon * A) / d$$

The constants ε represents the specific membrane capacitance (9fF/µm²), the constants d is the distance between the two layers of phospholipids, and A is the surface membrane area [203, 204].

Statistical analysis

Probability levels of random differences were determined by analysis of variance followed by Tukey-Kramer's multiple comparisons test and where applicable unpaired Student's t-test. Results were expressed as means ± SEM. $P < 0.05$ was considered statistically significant.

Results and Discussion

Paper I- Defining G protein-coupled receptor peptide ligand expressomes and signalomes in human and mouse islets.

GPCRs are seven transmembrane receptors that can bind external ligands (proteins, polypeptides or small molecules) to initiate effect inside the cell. In human islets, GPCRs play an important role in regulating the secretion of insulin, glucagon and somatostatin [82]. Some GPCRs ligands are expressed by the islet cells themselves as glucagon and somatostatin, acting as a regulator of glucose homeostasis while others are expressed and secreted by cells outside the islets and transported later by the systemic circulation such as the incretin hormones GLP-1 and GIP [205]. Human islets express 293 GPCRs and each of them can be a promising target for T2D treatment, however, the only islet GPCR that have a pharmaceutically available drug is GLP-1 receptor [206]. Most published studies have investigated the role of islet-derived peptide ligands on β-cell function in rodent. Nevertheless, till today these conducted researches did not address whether the results from mouse models are translatable to the human setting providing the fact that rodent and human islets show distinctive gene expression profiles in some respects [207, 208]. It is well known that some of the GPCRs mRNAs expressed by mouse islets are absent or expressed at only trace levels in human islets; thus questioning the translatability of those studies to the human context [209]. This was the reason to identify GPCR peptide ligand transcripts and GPCR/peptide signalling pathways which are common to mouse and human islets to facilitate the translation of mouse islets studies to the human islets.

Of the 159 human and 147 mouse GPCR peptide ligand genes, we detected the mRNAs encoding 128 in human islets while only 111 and 88 in the outbred ICR and inbred C57 mouse islets. Two of the most abundant GPCR peptide ligands identified in human and mouse islets are genes encoding glucagon and somatostatin. However, lower expression levels for islet amyloid polypeptide (IAPP) were detected in human islets although it was highly expressed in mouse islets. The reason for such differential expression pattern might be a greater proportion of α-cells in human islets compared to mouse islets since Iapp being synthesised and co-

stored with insulin from β-cells, which constitute 80% of the endocrine cells of mouse islets [210]. Similarly, the fifth most abundant GPCR peptide ligand gene in both C57 and ICR mouse islets, peptide YY (Pyy), was not highly expressed by human islets. Furthermore, PPY and UCN3 are two of the top ten expressed GPCR peptide ligand genes in both human and mouse islets, which propose the close species correlation in islet expression of abundant secretory peptide mRNAs. Expectedly, islets from both C57 and ICR mouse sharing nine out of ten of the most abundantly expressed GPCR peptide ligand genes (Col4a1, Gcg, Iapp, Pdyn, Ppy, Pyy, Rspo4, Sst, Ucn3), show great similarity in secretome profiles. On the other hand, islet mRNA expression of all GPCR peptide ligands indicated a lower correlation between human islets and the two mouse strains in comparison to the inter-strain correlation.

With further exploration, we were able to verify over 40 mRNAs encoding human GPCR peptide ligands, which were expressed above trace level in mouse islets in both C57 and ICR, and 42 mRNAs were quantifiable in human islets but absent or detected at trace levels in the mouse strains. On the contrary, 21 mRNAs encoding GPCR peptide ligand genes were undetectable in human islets but expressed above trace level in mouse islets. The most abundant mouse islet-specific GPCR ligand mRNAs are R-spondin-4 (Rspo4), prodynorphin (Pdyn) and the chemokines Ccl27a, Cx3cl1 and Ccl27b.

With the defined 418 peptide ligand/GPCR signalling pathways in human and mouse islets, we were able to generate signalomes atlas. In this atlas, several pathways for peptides that are synthesised and secreted from islets are demonstrated. These peptides interact with local islet GPCRs in an autocrine or paracrine manner, indicating the important effects of these peptides through activation of the islet GPCRs. Some pathways act as an intra-islet signalling pathway, where mRNAs encoding both the peptide ligand and its cognate receptor being expressed by islets. Other less common signalling extra-islet pathways, where cognate GPCRs for peptide ligands synthesised by islets, are not expressed in the islets. Species differences in mRNA expression were also identified by comparison of relative expression of GPCR peptide ligand and GPCR mRNAs, which is an indicative of the relative importance of particular pathways. As an example, very low levels of mRNA encoding C1QL1 was expressed in mouse islets contrary to the high levels expressed by human islets. Similarly, CXCL8 gene that encodes the chemotactic peptide IL-8 was abundantly expressed by human islets and absent in the mouse genome although mRNAs encoding the CXCR1 and CXCR2 receptors, through which IL-8 signals, are not expressed in the human islets. This could be due to an extra-islet role of the chemotactic peptide IL-8, following its secretion from human islet cells.

In this study, we also were able to detect a great difference in the expression levels certain GPCRs, which could predict the relative importance of particular pathways in mouse and human islets. For example, mRNAs encoding the NPY receptor ligands *i.e.* peptide YY (Pyy) and pancreatic polypeptide (Ppy), were almost expressed at ~40,000-fold (Pyy) and ~400-fold (Ppy) higher levels in mouse islets than in human islets.

Staining of mouse and human pancreas sections by fluorescence immunohistochemistry, indicated that PYY protein is present in both; mouse and human islets, with more intense immunostaining in mouse islets. When antibodies against insulin, glucagon and somatostatin were used and co-stained with PYY protein, results showed that the latter is primarily co-localised with α-cells; however, low expression was also detected in β and δ-cells. On the other hand, blocking NPY1R, which is a Gi-coupled receptor with the capability of inhibiting insulin secretion in both mouse and human islets [211], by BIBO 3304 trifluoroacetate (BIBO) results in blocking the effects of PYY released from islet cells in response to L-arginine. While insulin secretion from mouse and human islets in response to L-arginine was not affected by BIBO 3304 trifluoroacetate, glucagon and somatostatin secretion was increased only in mouse islets. In addition, two genes that are present at high levels in mouse islets, the Pdyn gene and the small secreted protein R-spondin, are absent or only found at trace levels in human islet. Our created signalomes showed that complement component 1, q subcomponent-like 1 (C1QL1) and adenylate cyclase activating polypeptide 1 (ADCYAP1) were expressed at much higher levels in human islets compared to mouse islets.

In summary, the current study shows the similarly and differentially expressed GPCR peptide ligand genes in mouse and human islets revealing the translatability of mouse studies to human.

Conclusion

1. A detailed signalome atlas of islet GPCR/peptide ligand was created highlighting the similarities and differences in GPCR peptide ligand expression and signalling pathways.
2. Glucagon, somatostatin and IAPP mRNAs were among the highly expressed transcripts in both human and mouse islets although IAPP transcript was comparably lower.
3. Islet-derived PYY have an important role in mouse rather than human islets, confirmed by functional studies with an NPY1R antagonist.

Paper II- Adhesion G Protein-Coupled Receptor G1 (ADGRG1/GPR56) and Pancreatic β-Cell Function.

In the current paper, we wanted to study the impact of ADGRG1 activation by its naturally accruing ligand *i.e.* Coll type III, on the pancreatic β-cell function. In addition, we also searched for the possible correlation of ADGRG1 with the other important genes for human islet function.

We found that ADGRG1 is the most abundantly expressed GPCR mRNA in isolated human pancreatic islets analysed by RNA sequencing and also confirmed by qPCR. ADGRG1 transcript was even more abundant than transcripts encoding the α_{2A} and GLP-1 receptors in human islets. Furthermore, we found different splice variants of ADGRG1 in human islets. ADGRG1 expression was negatively correlated with HbA1c. Islets from T2D donors showed a lower expression of ADGRG1 mRNA compared to nondiabetic islets, associated with a reduced insulin secretory response to glucose. Islets from *db/db* mouse (a diabetic animal model) displayed lower expression of Adgrg1. These data indicate an important role for ADGRG1 in β-cell function. Since ADGRG1 is expressed at similar levels in human, mouse and in rat insulinoma INS-1 832/13 cells as shown by different techniques, the further use of either of these cell systems would be translatable. We also studied the co-localization of ADGRG1 with insulin in human and mouse islets by confocal microscopy, which revealed a strong co-localization. Although basal secretion was increased, glucose-stimulated secretion was reduced in those *db/db* mice. Interestingly, human islets from untreated T2D donors show similar phenomena of a close correlation between a reduced ADGRG1 level and a reduced GSIS. These results show that the reduced ADGRG1 level can be a contributory factor to the pathophysiology of β-cell dysfunction in man.

Chronic hyperglycaemia is associated with a negative impact on β-cell function in T2D; therefore, we examined the effect of long-term culture at high glucose (20 mM) on the ADGRG1 expression in human islets. Our data showed that while short-term culture (24h) increases, long-term culture decreases the expression of ADGRG1 in human islets implicating the possible negative effects of glucotoxicity on the ADGRG1 expression. This reduced expression was associated with a dramatic reduction in cellular viability and increased apoptosis. In the present paper, we also showed that ADGRG1 is expressed by β-cells while Coll type III is not expressed and seems to be harboured from other cells of non-endocrine origin.

ADGRG1 down-regulation or loss-of-function was found to adversely affects normal organ development in the early embryonic state, suggesting a role for ADGRG1 in cellular differentiation and organogenesis [88]. To simulate ADGRG1 down-regulation, we used a cocktail of lenti virus in mouse islets, which were incubated for 60 minutes at low (1 mM) or high (16.7 mM) glucose after Adgrg1-

KD. These islets exhibited a reduced insulin secretory ability concomitant with a marked reduction in cAMP production in response to Coll type III. Since the islet cAMP producing capability in response to forskolin or GLP-1 (two known cAMP enhancing agents) was not affected, we could conclude that the impaired cAMP generation in response to Coll type III, upon Adgrg1-KD, is a specific phenomenon. The reduced cAMP generation in Adgrg1-KD was also reflected in the reduced PKA activity. Taken together, the data suggest that the beneficial effect of Coll type III is mediated via activation of Adgrg1 with subsequent positive impact on the cAMP/PKA system.

Furthermore, as mentioned above, we also showed that Adgrg1 receptor protects β-cells against apoptosis since its down-regulation initiate apoptosis as shown by the cleaved PARP and increased RhoA phosphorylation in INS-1 832/13. RhoA is known to be a key regulator of β-cell dysfunction and it signals via its effector Rho-associated, coiled-coil containing protein kinase 1. Coll type III was able to inhibition of the RhoA signalling pathway when added to the culture media. Similarly, Coll type III had a suppressive effect on caspase-3 activity when the INS-1 832/13 cells were cultured at 5 or 20 mM glucose for 72h. Next, we investigated cell apoptosis by measuring cytoplasmic nucleosomes in INS-1 832/13. In scramble control cells, the rate of apoptosis was very low at 5 mM glucose whilst there was a marked increase in cell apoptosis at 5 mM glucose upon receptor down-regulation, and apoptosis was further increased with culturing the cells at 20 mM glucose. Coll type III did not suppress apoptosis when Adgrg1 was down-regulated.

Does Coll type III potentiate insulin secretion in human islets? We set to test the effect of Coll type III on insulin secretion in isolated human islets. Similar to the data obtained using mouse islets, we found that Coll type III greatly potentiates glucose-stimulated insulin secretion in islets from nondiabetic and T2D human islet donors. These results suggest that targeting ADGRG1 might be a new approach for the treatment of T2D (figure 8).

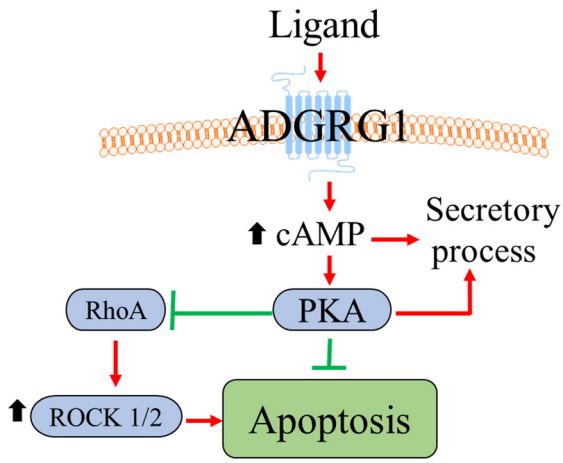

Figure 8. Schematic representation of the signalling pathway exerted by ADGRG1/GPR56 upon activation by Coll III. The positive impact on cAMP/PKA system potentiates insulin secretion and reduces apoptosis in the β-cell.

Conclusion

- The beneficial effect of Collagen type III on β-cell is mediated through activation of ADGRG1.
- ADGRG1 has a great correlation with the expression of almost 15000 transcripts demonstrate the importance of ADGRG1 for a proper β-cell function.
- Activation of ADGRG1 results in the activation of cAMP/PKA signalling system.
- ADGRG1 activation is associated with increased β-cell viability, adhesion, suppression of apoptotic signals and potentiation of glucose-stimulated insulin release.

Paper III- Preserving Insulin Secretion in Diabetes by Inhibiting VDAC1 Overexpression and Surface Translocation in β Cells

Insulin exocytosis is dependent on normal functioning mitochondria, where glucose oxidation takes place to generate ATP. In turn, ATP increases cytosolic Ca^{2+} in a complex process leading to insulin secretion. This signalling cascade is impaired in T2D, mainly due to defective mitochondrial metabolism [212, 213].

VDAC which is located in the outer mitochondrial membrane represents the most abundant protein which functions as a regulator of the flux of ions, metabolites, nucleotides such as ADP and ATP between the mitochondria and the cytosol. Three isoforms of VDAC exist; VDAC1 and VDAC2, which determine cell life and death while the less well characterized one is VDAC3 isoform [119]. VDAC1 is extensively studied in relation to Alzheimer's disease, where VDAC1 is found to be induced early in the disease and the presence of VDAC1 antibodies strikingly protect cells from neurotoxicity induced by Aβ peptide [132, 214]. Recently more studies toward the association between T2D and Alzheimer's disease have been conducted, but none had investigated the involvement of VDAC in β-cell glucotoxicity.

Therefore we measured VDAC expression in islets of ND and T2D donors. We found that T2D Islets displayed altered VDAC expression, whereas VDAC1 mRNA is upregulated, and VDAC2 mRNA is repressed. This upregulation of VDAC1 mRNA is correlated with HbA1c in ND islets, and the same correlation was seen in T2D donors, but less markedly, although still significant. We speculated that the difference in correlation between ND and T2D could be related to the fact that four of these T2D donors where on metformin treatment, where those islets did not display increased VDAC1 mRNA. Conversely, VDAC2 mRNA showed a negative correlation with HbA1c and the removal of the metformin-treated donors only marginally changed that expression. We also identified two abundantly expressed VDAC1 transcript variants, where one of them appears to be induced by hyperglycaemias, as it was already somewhat increased in islets from donors with impaired glucose tolerance, suggesting the role of elevated blood glucose in VDAC1 overexpression. To simulate glucotoxic conditions, we cultured human islets under 20 mM glucose for 72h, which resulted in the increased VDAC1 and decreased VDAC2 mRNA expression. Nevertheless culturing at an intermediate glucose concentration (10 mM) for 72h did not significantly affected VDAC1 mRNA compared to the basal culture medium (5 mM), while it negatively affected VDAC2 mRNA expression. It seems that VDAC2 suppression is more sensitive to elevated glucose contrary to VDAC1, which requires longer culturing time at high glucose. Interestingly, the inclusion of 20 μM metformin in the culture medium

prevents the alterations in VDAC1 and VDAC2 mRNA levels confirming the observations seen in metformin-treated donors' islet.

VDAC2 has an important role in β-cells function provided by the finding that VDAC2 protein is more abundant than VDAC1 in INS-1 cells. Therefore we investigated the effect overexpression VDAC1 and silencing VDAC2 in INS-1 cells and human islets. It was established that overexpression of VDAC1 suppresses VDAC2, and silencing of VDAC2 increases VDAC1, meaning that altered expression of either isoform could impact on β-cell function. VDAC1 upregulation is known to be caused by metabolic and oxidative stress resulting in cellular apoptosis. We confirmed this finding by measuring cytoplasmic nucleosomes in INS-1 cells when Vdac1 is overexpressed or Vdac2 is knockdown at 5 mM and 20 mM glucose, where the later concentration results in marked cell death. Similarly, GSIS is markedly inhibited after Vdac1 overexpression or Vdac2 knockdown in INS-1 cells.

The need to investigate the role of these two VDAC isoforms in β-cell function specified assessing mitochondrial function at glucotoxic conditions. OCR is inhibited both at non-stimulatory and stimulatory glucose concentrations in cells with altered Vdac isoform expression and a similar inhibition was seen also when the cells were cultured under glucotoxic conditions. Both cytosolic and mitochondrial Ca^{2+}evoked by glucose are markedly blunted in cells with upregulated VDAC1 or downregulated VDAC2, as well as after glucotoxic treatment which is expected due to the attenuated GSIS. VDAC1 is essential in the function of ER-mitochondrial contact sites [215], where it was observed that human T2D β-cells revealed decreased VDAC1 protein in these contact sites. Denotation that both; the altered VDAC gene expression and protein localization are responsible for the mitochondrial dysfunction. But does silencing VDAC1 protect human islets from glucotoxicity? To evaluate this, we measured overall cellular reductive capacity, reflecting β-cell mitochondrial metabolism and we found that VDAC1 knockdown did not alter reductive capacity in 5 mM glucose-cultured islets whilst it completely prevents the marked impairment under the glucotoxic condition contrary to VDAC2 silencing, which induced low overall cellular reductive capacity similar to 20 mM glucose culture. These results were validated by measurements of ATP content and insulin secretion after 1h incubation of islets after prior culture at either 5 or 20 mM glucose. VDAC1 Silencing not only preserves basal ATP levels, but also rescues glucose-induced increases in ATP and insulin secretion, contrary to VDAC2 suppression, which reduces basal islet ATP and blunts both ATP elevation and stimulated insulin secretion. These results indicate that mitochondrial metabolism is impaired by the altered VDAC expression profile in glucotoxic conditions resulting in impairment of GSIS, while the impact on β-cell viability is less pronounced.

Glucotoxicity is known to upregulate TXNIP in the β-cell by the induction and nuclear transfer of the carbohydrate response element-binding protein (ChREBP) [216]. Similarly, the non-metabolizable glucose analogue 2-deoxyglucose stimulates metabolism-independent nuclear transfer of ChREBP. We found that T2D islets display a similar increase of both the transcripts of ChREBP and TXNIP confirming published results.

Next, we wanted to investigate the cellular localization of VDAC1 and VDAC2 by confocal microscopy. We found that β-cells from T2D donors exhibit VDAC1 surface expression which was not seen in both; ND and in the single donor that we examined out of four with documented metformin therapy. This surface expression of VDAC1 correlates positively with the islet donor HbA1c when ND and T2D islets were pooled. High plasma glucose concentrations in these donors could therefore, be the cause for VDAC1 surface localization. We carried on with triple immunofluorescence staining in pancreas sections of ND and T2D donors, which clearly demonstrated that VDAC1 is overexpressed at the T2D β-cell surface. Similarly, an overexpressed VDAC1 was seen in β-cell of ND islets kept in culture for 72h at 20 mM glucose relative to control conditions. Likewise, INS-1 cells cultured under glucotoxic conditions displayed VDAC1 expression at the cell surface. These results indicate that VDAC1 mistargeting to the plasma membrane may cause the impaired GSIS in glucotoxicity and T2D. VDAC1 mistargeting was also reported in AD by Fernandez-Echevarria *et al*, where the neurons of the affected brain areas have an overexpressed VDAC1 associated with translocation of the protein to the neurolemma [214].

But what are the functional consequences of aberrant VDAC1 subcellular localization? It is well documented that various mouse and human tissues display plasma membrane-resident VDAC1 with the mitochondrial surface residues facing the extracellular space. We hypothesized that VDAC1 overexpression leads to its targeting to extra-mitochondrial locations. The mouse Vdac1 gene is alternatively transcribed, yielding an exon1 splice variant encoding a plasma membrane-targeted protein (plVDAC1) [115]. Human and rat VDAC1 genes lack such splicing. Activation of plVDAC1 in neurons was found to initiate apoptosis, which is prevented by antibodies directed against the extracellular N terminus of VDAC1 [132]. We investigated similar conditions in INS-1 cells, with induced glucotoxicity-evoked VDAC1 induction. Remarkably, not only VDAC1 antibody, VBIT-4 and AKOS022075291 (AKOS), but also metformin prevented the glucotoxicity-mediated overexpression of VDAC1 mRNA [217] . Furthermore, culturing ND islets at 20 mM glucose with the inclusion of VBIT-4 also decreases VDAC1 protein expression. Therefore, overexpression of VDAC1 is prevented by metformin and inhibitors of VDAC1, suggesting that, as in neurons [218] plVDAC1 conductance is harmful and notably affecting gene transcription in β-cells. It is worth mentioning that VDAC1 overexpression was not noticed in the limited

number of T2D donors with documented metformin therapy suggesting protective action of metformin by preventing the high glucose-evoked changes in VDAC1 and VDAC2 expression in islets and INS-1 cells.

Next, we wanted to investigate the consequence of VDAC1 localization in the cell membrane. To this end, we monitored ATP levels during 1h experiments in plVdac1-expressing INS-1 cells. The results showed that overexpression of WT Vdac1 (mt Vdac1) leads to a 3-fold increase in ATP release from the cells, whilst plVdac1 expression causes a 10-fold ATP loss. In parallel, the cells transfected with mtVDAC1 show a marked reduction in GSIS, which was even completely abolished in plVdac1-expressing cells. Moreover, cell death is aggravated in mtVdac1 cells in 20 mM glucose, while plVdac1 alone is already harmful at 5 mM glucose culture. The acute and rapid loss of ATP during 1h is inhibited by VDAC1 antibody, VDAC inhibitors AKOS and VBIT-4. Of note, metformin also efficiently inhibited the depletion of ATP. It can be concluded that ATP loss is due to VDAC1 overexpression and translocation to the plasma membrane. Furthermore, INS-1 cells overexpressing plVDAC1 displayed 30% higher membrane conductance, as measured by whole-cell patch-clamp recording. This increase in conductance was abolished by the acute addition of either VDAC1 antibody or metformin. Control INS-1 cells were not affected by superfusion with metformin. As mtVDAC1 and pl-VDAC1 display identical amino acid sequences [119], we used purified mitochondrial VDAC1 protein to further study metformin action. For this purpose, we recorded the channel conductance with purified mitochondrial VDAC1 protein reconstituted into planar lipid bilayers. Metformin like the specific VDAC inhibitor VBIT-4 decreased the conductance to a low conducting state. In T2D, VDAC1 mistargeting to the cell surface may involve post-translational modification of its only two cysteine residues (cys127/232) [219]. Therefore, we used cysteine-deleted VDAC1 plasmid transfection in INS-1 cells, cysteine-depleted Vdac1 is more overexpressed than mtVdac1. Although higher in expression, cysteine-depleted Vdac1 plasma membrane near localization is 50% less than mtVdac1. The increase of the cellular ATP/ADP ratio by glucose stimulation is largely preserved in the mutated VDAC1-overexpressing cells and there was no increase in ATP release in contrast to mtVDAC1- expressing cells. Furthermore, reciprocal suppression of Vdac2 observed after mtVDAC1 overexpression is absent. All these observations explain the near normal GSIS in the cells transfected with the mutant VDAC1. These results strongly suggest that posttranslational modification of the two cysteine residues in VDAC1 is involved in the mistargeting to the plasma membrane.

Diabetic *db/db* mice β-cells also showed surface expression of VDAC1 similar to islets from T2D donors contrary to normoglycemic C57/bl mice. This increase in surface expression is associated with increased Vdac1 mRNA. In islets of hyperglycemic *db/db* mice, glucose (16.7 mM) increased ATP release but failed to evoke the normal ATP increase. The presence of VDAC1 antibody or metformin

reduces ATP release and increases its cellular levels resulting in enhanced ATP generation and GSIS. Normoglycemic C57/bl mouse islets were not affected by metformin or VDAC1 antibody treatment, confirming that VDAC1 mistargeting is restricted to diabetic β-cells. Moreover, although ATP content and cellular reductive capacity were not affected by the inclusion of metformin, VDAC1 antibody or VDAC1 inhibitors after culture of ND islets at 5 mM glucose whilst they markedly improved metabolism during glucotoxic conditions. Similar results were obtained from T2D and not in ND islets, where the attenuation of GSIS observed in the islets cultured at 20 mM glucose was prevented after inclusion of VDAC1 antibody or metformin in the culture medium. This effect was associated with prevention of VDAC1 overexpression by glucotoxicity. Next, we queried if the defective stimulus-secretion coupling in T2D islets could be improved by acute exposure to the VDAC1 inhibitors. Most remarkably, T2D islets showed increased total islet ATP content by 5-fold in both at 1 and 16.7 mM glucose after only 1h exposure to VDAC1 antibody, VBIT-4, AKOS or metformin. Concomitantly, there was a nearly 4-fold increase in GSIS compared to the merely 10% augmentation in the absence of the inhibitors. This is strong evidence that VDAC1 mistargeting underlies the impaired β-cell function.

By now, we were encouraged to study the effects of the VDAC1 inhibitor VBIT-4 in vivo. Young *db/db* mice, starting at age 42 days were subjected to daily intraperitoneal injections of VBIT-4 for 5 weeks. While the vehicle-injected *db/db* mice rapidly developed severe hyperglycaemias, VBIT-4 completely prevented the increase in blood glucose. Discontinuing the treatment resulted in a gradual increase of blood glucose concentrations over several weeks, reaching those of vehicle-treated animals. As such a slow return of hyperglycaemia is not seen after discontinuation of other anti-diabetic drugs, it is likely that inhibition of VDAC1 has disease-modifying actions. Of note, contrary to *db/db* mice, control C57/bl mice did not show any changes in blood glucose by this treatment. Comparing our results in *db/db* mice with published work [220], it appears that VBIT-4 is more efficient than metformin in preventing the increase in blood glucose when the treatment is initiated prior to the development of hyperglycaemia. This is possibly explained by more efficient binding of VBIT-4 to VDAC1 compared with metformin. To summarize, early initiation of therapy with a VDAC1 inhibitor blocks the development of diabetes in this T2D mouse model. Figure 9 summarizes the events related to glucotoxicity associated increase in the VDAC1 expression and mistargeting to the cell membrane with a subsequent effect on the β-cell ATP handling.

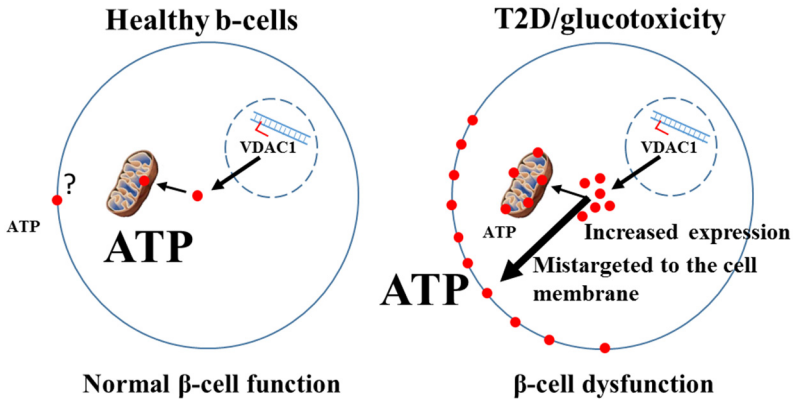

Figure 9. Schematic representation of the β-cell dysfunctionality under glucotoxic condition showing the increased VDAC1 expression with subsequent mistargeting to the cell membrane. The reduced insulin secretory capacity is associated with ATP loss and decreased β-cell viability.

Conclusion

- VDAC1 is mistargeted to the β-cell plasma membrane in glucotoxicity as well as in islets of T2D donors.
- VDAC1 surface mistargeting leads to β-cell ATP loss, impaired insulin secretion and eventually decreased β-cell viability.
- Specific VDAC1 blockers slow diabetes progression by preventing VDAC1 induction and ATP loss.
- Metformin also inhibits VDAC1, which adds a novel mode of action for this anti-diabetic drug.

Paper IV- Adhesion G Protein-Coupled Receptor G1 (Adgrg1) activation counteracts pancreatic β-cell dysfunction

The ADGRG1/GPR56 receptor is involved in several biological and physiological processes within the cells hence it is implicated in many important cellular functions [221]. This adhesion GPCR regulates secretion of insulin and harmonizes different intracellular signalling pathways in the β-cell preserving its structure and viability. In the paper 2, we showed that Adgrg1 activation increases the activity of cAMP/PKA system in β-cells, although, it has been reported that it might activate the Gαq signalling cascade which has been shown in neuronal cells [222]. Nevertheless, its impact on the other important signalling pathways for β-cells such as MAPks and the major transcriptional factors such as NFkB, STAT3 or STAT5 has not been established.

In the current paper, we investigated the possible impact of Adgrg1-KD on the activation/inhibition of major signalling pathways in the β-cells. Our results showed that, when calculated as % of content, GSIS seems not to be affected by Adgrg1-KD, while separately analysis of the data showed that both GSIS and insulin content is reduced in Adgrg1 cells. Thus the appropriate way of presentation of GSIS might mask the important effect of Adgrg1-KD on the insulin secretory response of the β-cells. This seemingly released insulin might be in fact an insulin leakage and not a true exocytosis, due to loss of cellular structure and adhesion property caused by Adgrg1-KD. To validate the results, we used diazoxide, an inhibitor of insulin release acting through the opening of the K^{+}_{ATP} channel. In the scramble control INS-1 cells, GSIS was markedly suppressed by diazoxide without any influence on the insulin content. In contrast, there was no attenuation of the already reduced insulin secretion by diazoxide in Adgrg1-KD INS-1 cells.

Since we have shown (paper II) that Adgrg1-KD affects cellular structure, reduces mitochondrial function and causes β-cell dysfunction, similar to diabetic β-cells, we were curious to study the possible involvement of newly recognized transcriptional factors as well as mitochondrial anion channels, associating with β-cell dysfunction (paper III) in Adgrg1-KD induced β-cell dysfunction, in view of our observations in glucotoxic conditions and in diabetic islets. We found that Adgrg1-KD is associated with attenuation of Coll type III-induced suppression of P70S6K, JNK, AKT, NFkB, STAT3 and STAT5 phosphorylation/activity. The suppression of these signals, which normally are associating with β-cell dysfunction, by Coll type III, is further proved on the beneficial effects of Coll type III on β-cell function. Contrary to these signals, Coll type III increased the activation of CREB in scramble control INS-1 cells, regardless of the ambient glucose levels, although this effect was totally abolished in Adgrg1-KD INS-1 cells cultured at 5 mM glucose but not

in the presence of 20 mM glucose. It seems that Adgrg1-KD forces the activation of the above MAPKs and the measured transcriptional factors, which are associated with detrimental effects on the β-cell function and might explain the observed apoptosis upon Adgrg1-KD (paper II).

We also showed that *Chrebp* and *Txnip*, which are associated with glucotoxicity-induced β-cell dysfunction [223, 224], are both increased in Adgrg1-KD cells. Our results also showed that the mitochondrial gatekeeper VDAC1 is increased in the Adgrg1-KD cells. As we showed in paper III, the increased *Chrebp* and *Txnip* might similarly have an important impact on the Vdac1 expression in Adgrg1-KD cells. Adgrg1-KD cells displayed a reduced Vdac2 expression which could be explained by the fact that when Vdac1 is upregulated, it negatively impacts Vdac2 expression (paper III).

VDAC1 has been linked to neuronal cell destruction in Alzheimer's disease, where increased expression of VDAC1 triggers cell death. We have demonstrated that such a scenario is operative in insulin producing β-cells in T2D (paper III) and could therefore also occur in Adgrg1-KD (current paper). Therefore, we investigated VDAC1 protein expression in isolated mouse pancreatic islets by confocal microscopy. Adgrg1-KD markedly increased VDAC1 protein expression compared with very low expression in scramble control islets. Strong co-localization of VDAC1 with the plasma membrane-associated SNARE protein SNAP-25 (cell membrane marker) was also detected indicating VDAC1 surface expression. These results were confirmed further by finding that Adgrg1-KD cells were associated with a marked ATP release contrary to scramble control cells. The presence of Coll type III in the incubation medium was unable to suppress the enhanced ATP release from Adgrg1-KD cells, whilst VBIT-4 or AKOS (the inhibitor of VDAC1) greatly suppressed it. This was a strong confirmation of the previous results and further emphasizing the deleterious effect of plasma membrane expression of VDAC1 and the loss of ATP, since VDAC1 is ATP-conducting anion channel allowing the transport of ATP from mitochondria to the cytoplasm in the normal β-cells. Thus, Adgrg1-KD is associated with transcriptional impact increasing VDAC1 expression, followed by mistargeting of VDAC1 to the plasma membrane in β-cells with the consequent loss of metabolites such as ATP. Taken together, activation of Adgrg1 by Coll type III is associated with suppression of major mitogen-activated protein kinases and transcriptional factors probably via activation of the cAMP-PKA-CREB signalling pathway. Our data also support the involvement of an altered VDAC1/VDAC2 expression in the impaired GSIS evoked by Adgrg1-KD.

Conclusion

- Adgrg1-KD is associated with increased phosphorylation/activation of the major inflammatory signalling pathway operative in the dysfunctional β-cell.
- Adgrg1-KD is accompanied by with mistargeting/translocation of mitochondrial VDAC1 to the cell membrane in INS-1 cells resulting in an increased loss of ATP.

Paper V- The functional impact of G protein–coupled receptor 142 (Gpr142) on pancreatic β-cell in rodents.

GPR142 is one of the reasonably expressed receptors among the identified GPCRs in pancreatic islet [82]. Recent studies have shown that GPR142 positively affect GSIS in mouse islets, which has attracted interest as a novel target for treatment of T2D [95-98]. Therefore, we wanted to investigate the functional impact of Gpr142 in more detail in rodent islets and in rodent β-cell line *i.e.* INS-1 832/13 cell. In this paper, we showed that Gpr142 is more expressed in β-cells than glucagon producing α-cells or somatostatin producing δ-cells. This reveals that in addition to β-cells, Gpr142 agonists might exert a functional impact on the certain populations of α- or δ-cells. It is difficult to explain why Gpr142 is not equally expressed in all glucagon- or somatostatin-expressing cells, but it should be kept in mind that there could be a functional heterogeneity among the cells of the same category as it has been highlighted previously [225]. From a pharmacological point of view, highly potent GPR142 agonists might also stimulate glucagon and somatostatin secretion concerning T2D subjects who already display an uncontrolled increase of blood glucagon.

Both L-Tryptophan and L-Phenylalanine are the known endogenous ligand of GPR142, but in the present study, we used two synthetic GPR142 agonists (compound 33 and compound A) [226]. We found that both compounds potentiate GSIS in INS-1 832/13 cells, by increasing cellular levels of cAMP hence showing for the first time that the signalling pathway associated with Gpr142 activation involves Gs in rodent β-cell. Previous reports by Lin and colleagues had suggested that Gq is the associated downstream signal for the Gpr142 activation [97]. A possible explanation for the discrepant results in our and Lin *et al* studies could be due to the use of different cell lines since we used insulin producing INS-1 cells while they used HEK293 cells overexpressing Gpr142. It should be recalled that signalling pathway for a GPCR might differ in the cells with different origin [227],

although overexpression of Gpr142 in the cell might cause the interaction with more than one G-coupled proteins as has been shown for other GPCRs [227]. To functionally confirm our observed results of Gpr142 being associated with cAMP-PKA system in β-cells, we studied the effect of acute (60 min) blockade of Gpr142 on GSIS when cAMP-PKA system was inhibited by two specific inhibitors Myr-PKI (PKA) and ESI-05 (Epac) respectively and compared this effect with the inhibition of PLC (downstream effector of Gq activation). Our results showed that GSIS was markedly attenuated the in the presence of cAMP-PKA inhibitors while inhibition of PLC was less influenced. It should be recalled that, as expected, Bt_2-cAMP was able to potentiate insulin secretion in both control and Gpr142-KD cells. These results favour cAMP/PKA/Epac pathway being much more superior to that of PLC pathway concerning the selective Gpr142 activation.

Islets from T2D donors show increased expression of pro-inflammatory cytokines indicating β-cell dysfunction; therefore, we wanted to test for the presence of pro-inflammatory cytokines when the receptor is knock-down and whether the agonists can still potentiate insulin secretions in that case. To this end, we preformed knock-down of GPR142 in mouse islets and measured the release of cytokines during a 6-hour recovery period following the knock-down. The culture medium revealed a remarkable increase of the cytokines MCP-1, IFNγ and TNFα, which could be an additional factor in the attenuation of β-cell function seen after Gpr142-KD. These results agree with our RNAseq data showing a positive correlation of MCP-1 transcript with HbA1c in islets from IGT and T2D donors. Moreover, our results also indicate that Gpr142-KD is associated with the release of cytokines in the absence of any immunogenic stimuli. Subsequently tested Gpr142-KD islets showed a reduced GSIS and a complete abolished potentiation of GSIS by GPR142 agonists compared to scramble control islets.

Next, it was interesting to study whether Gpr142-KD has any impact on the expression of certain GPCRs with the known impact on the Gq and Gs. The results showed that receptors with distinct (Gq/o) coupling were not influenced whilst Gpr56, which has the (Gs) signalling function, is negatively affected. This could be a direct effect of Gpr142-KD or the consequence of the reduced cAMP level, evoked by the ablation of Gpr142 in INS-1 832/13 cells. The expression of *Tlr5* and *Tlr7*, two receptors involved in mediating inflammatory signals in β-cells, were markedly increased in Gpr142-KD cells. Moreover, we also found that Gpr142-KD is associated with a significant increase in the expression of the transcripts involved in β-cell dysfunction such as *Chrebp, Txnip, NFkB, RhoA* and *NOS2* (*iNOS*). Other transcriptional factors such as *Pdx1* and *Pax6* which are important in maintaining normal β-cell viability and function were reduced as a consequence of Gpr142-KD. These results highly support the importance of Gpr142 in preserving β-cell function and show that silencing the receptor results in cellular stress.

To mimic the impact of hyperglycaemia on β-cell, we exposed INS-1 832/13 cells to high concentration (20 mM) glucose for 72h in the presence or absence of Gpr142 agonists or a non-metabolized cAMP analogue (Bt_2-cAMP). Glucotoxicity reduced Gpr142 expression while the expression of *Chrebp, Txnip* and *Vdac1* mRNA was increased. The presence of Gpr142 selective agonists or Bt_2-cAMP during the culture period prevented the glucotoxic effect.

It was mandatory to assess mitochondrial function and measure apoptosis by cellular reductive capacity and cytoplasmic nucleosomes respectively in INS-1 832/13 cells to compare cellular viability between control and Gpr142-KD cells. Ablation of Gpr142 results in attenuated reductive capacity already at normal glucose levels (5 mM), which was further reduced in the presence of higher glucose concentration (20 mM). Apoptosis was increased at 5 mM glucose and further increased when cells were cultured at 20 mM glucose upon Gpr142-KD. Compound 33 did not prevent the glucotoxic effect on cell viability in contrast to the Bt_2-cAMP compound, which was able to prevent generation of apoptotic signals and enhance β-cell viability. This indicates that cAMP-activated signalling pathway is still operational in Gpr142-KD cells, the stimulation of which by other means or agents could exert protective effects in β-cell protection (Figure 10).

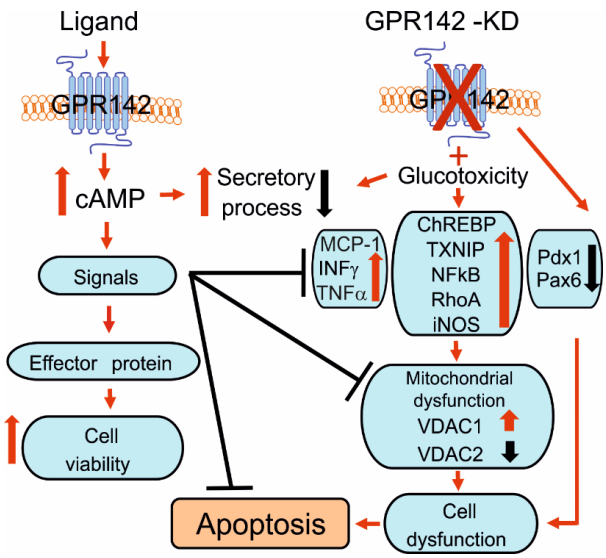

Figure 10. Gpr142 activation by its ligand with activation of cAMP-PKA signalling pathway. The resultant increase in β-cell viability is associated with increased secretory capacity and reduced pro-inflammatory cytokines.

Conclusion

1. Gpr142 is expressed in mouse islets, predominantly in β-cells and its activation increases GSIS via a cAMP-PKA pathway.
2. Basal activity of Gpr142 seems also to be important for the β-cells since Gpr142-KD is associated with increased expression of transcripts for transcriptional factors and enzymes with known negative effects on β-cells and GSIS.
3. Gpr142-KD also reduces β-cell viability and increases apoptosis via at least in part, increased expression of mitochondrial VDAC1.
4. GPR142 agonists have the ability to potentiate GSIS as well as suppress progressive inflammatory signals that spontaneously affect pancreatic islets and insulin producing β-cells, thus the GPR142 agonists might have an important therapeutic usefulness in the management of T2D.

Final Remarks

The major concluding interpretations from the current thesis are that despite the complexity of T2D, there are several ways to prevent or postpone the progression of metabolic disorders in the overt T2D. As it has been shown in the present thesis, both directly targeting and blocking the surface translocated VDAC1 in diabetic β-cells by small molecules or peptides, to prevent ATP loss, is a new way to prevent β-cell dysfunction and apoptosis. Furthermore, the identification of the altered VDAC1 signalling axis may have important consequences in other fields beyond β-cell dysfunction and diabetes where VDAC1 also has major regulatory roles, such as Alzheimer's disease and other neurodegenerative. Additional in vivo studies to test lead compounds, optimally inhibiting VDAC1 are required to test this hypothesis.

Since β-cell dysfunction in T2D β-cell might have different origins, we also present an additional target for the restoration of β-cell dysfunction. Among such additional targets are the GPCRs especially, the "orphan" one with no known ligand, which could be a new class of substances for the treatment of not only β-cell dysfunction but also disorders in other tissues. We show that, mitogenic as well as anti-apoptotic effects of two recently deorphanized GPCRs *i.e.* GPR56 (Adgrg1) and GPR142, which are strongly expressed on the β-cell membrane, could be a therapeutic approach for early stages of T2D treatment.

Future perspective

It has been established that perturbation of insulin secretion by pancreatic β-cells is the main defect leading to sustained hyperglycaemias, abnormalities of intermediary metabolism with subsequent progression into type 2 Diabetes (T2D) T2D. As the disease progresses, the capability of the β-cell to secrete sufficient amounts of insulin to face hyperglycaemias decreases, thereby leading to progression of hyperglycaemias which causes additional harm on the β-cells and other cells in the body.

The few frequently used antidiabetic drugs on the market *i.e.* metformin, sulphonylureas, GLP-1 analogues and DPPI, have not satisfactorily demonstrated any long-lasting improvements of β-cell function. This is the reason why many type 2 diabetic patients require insulin therapy after a few years of treatment.

It should be emphasized that there is a common and imminent feature in the medical treatment of human diseases that regardless of the treated disease, long-term monotherapy is often associated with the development of the body's desensitization or resistance to the drug used. An alternative to improving the secretory capacity of diabetic β-cells and to avoid the reduced sensitivity to a particular drug should prompt the development of drugs with different site/mechanisms of action.

Since the seven-transmembrane pass, receptor proteins also called G-protein-coupled receptors (GPCRs) are the largest family of receptors with a diverse and distinct mechanistic impact on the β-cell function, we believe that they represent a potentially interesting group of the target protein for future drug development (paper I). A couple of these GPCRs *i.e.* GPR56 and GPR142 that we present in this thesis (paper II, IV and V) play an essential role in mediating beneficial effects on the β-cell function. Thus, developing small molecule or peptide drugs with agonistic properties for these receptors is highly motivated to yield future drugs that can affect β-cells to regain their functionality in T2D patients.

Our studies showed a decisive impact of VDAC1 on the insulin cell function. An attractive and considerable extension of our study is to investigate the impact of VDAC1 on the peripheral insulin-sensitive cells. There are multiple consequences of long-lasting hyperglycaemias and diabetes on the other tissues. We will also extend these analyses to other organs such as liver, endothelial, kidney and fat cells to investigate the possible impact of VDAC1 overexpression and mistargeting to the

cell surface on the development of diabetic complications. If our observations from the pancreatic β-cell are burned out and extended to other tissues, it may help in treating or preventing diabetic complications (eg cardiovascular and renal failure).

Populärvetenskaplig sammanfattning

Hormonella signalsystem är viktiga för att bibehålla balansen i kroppen. Störningar i detta system ger upphov till ett antal sjukdomar inklusive diabetes. Diabetes är en sjukdom där blodsockret är för högt. Sockerhalten i blodet ökar när vi äter. Sockret används sedan som energikälla för kroppens celler. För att cellerna ska kunna ta upp och använda sockret behövs ett hormon som heter insulin. Insulin produceras och frisätts från β-cellerna i de Langerhanska öarna i bukspottskörteln. Om insulin inte frisätts i tillräcklig mängd för att möta kroppens behov riskerar vi att utveckla diabetes.

Man kan grovt dela in diabetes i två huvudtyper; typ 1-diabetes och typ 2-diabetes. Typ-1 diabetikern saknar insulinproducerande β-celler. Typ 2-diabetikern däremot har insulinproducerande cellerna men dessa klarar inte av att frisätta tillräckligt med insulin. De studier som presenteras i min doktorsavhandling rör framförallt typ-2 diabetes och är inriktade på att undersöka orsaken till varför β-cellerna inte fungerar vid typ 2-diabetes samt att hitta sätt att återställa β-cellernas insulinfrisättande förmåga.

En receptor är ett protein som fungerar som en mottagare för signaler som skickas i kroppen. G-proteinkopplade receptorer (GPCR) är en typ av receptorer som sitter på cellens yta. De är mycket intressanta för forskningen eftersom de utgör ett viktigt mål för många läkemedel. I det första arbetet har vi jämfört genuttrycket genom mätning av mRNA som kodar för kända peptid-signaler (ligander) som binder till och aktiverar GPCR i Langerhanska öar från människa och mus. Vi skapade på detta sätt ett slags detaljerat uppslagsverk (Atlas) för olika GPCR och deras ligander. Detta uppslagsverk kan användas för att förstå likheterna och skillnaderna i GPCR/ligand-uttryck i mus och människa. Informationen kommer att hjälpa forskare att fokusera på forskning runt GPCRs som är relevanta och viktiga för att behandla diabetes hos människan.

I Arbete 2 och 4, som delvis hänger ihop, har vi använt informationen som vi tog fram i arbete 1 för att studera en GPCR som det finns mycket av i insulinproducerande celler från både mus och människa; nämligen GPR56 (ADGRG1). I detta arbete fann vi att ämnen som aktiverar denna receptor (sk agonister) skulle kunna fungera som läkemedel för behandling av typ 2 diabetes.

Typ 2 diabetes utvecklas efter år av prediabetes. Vid prediabetes har individen ett kontinuerlig högre blodsocker halt jämfört med normala individer. Högt blodsocker under en lång tid fungerar som ett gift på β-cellerna och försämrar dessas förmåga att frisätta insulin. Detta fenomen kallas glukotoxicitet (glukos=socker). Vi rapporterar i arbete 3 att mängden av ATP-transporterande yttre mitokondriella membranspänningsberoende anionkanal-1 (VDAC1), ett protein som finns inne i de insulinproducerande β-cellerna, är högre i β-celler från organdonatorer som led av typ-2 diabetes än friska kontroller. På samma sätt är mängden VDAC1 högre i β-celler från friska donatorer när cellerna odlats länge i höga sockernivåer (glukotoxiska förhållanden). Vi tror att om β-celler utsätts för högt blodsocker, t.ex. vid prediabets så får de felaktigt höga nivåer av VDAC1. När mängden VDAC1 blir alltför hög kommer en viss mängd av proteinet att hamna på cellens yta där det normalt inte hör hemma. När VDAC1 finns på cellytan fungerar det som en dörr genom vilket ATP, cellens energimolekyl, kan ta sig ut ur cellen. Enkelt uttryckt kan man säga att cellen läcker energi på grund av att VDAC1 är på fel ställe. Förlusten av energi (ATP) gör att β-cellen inte kan fungera optimalt och detta på sikt kan leda till att β-cellerna dör. Vi har kunnat visa att om man förhindrar att cellen läcker ATP genom fellokaliserade VDAC kanaler på cellytan, så kan man återställa insulin frisättningen i β-celler från diabetiska organdonatorer. På samma sätt kunde vi ”bota” prediabetiska möss genom att behandla de med en VDAC1-hämmare och förhindra att cellerna läckage av ATP. Därigenom kunde mössen skuddas från att utveckla diabetes.

I Arbete 5 undersökte vi en annan GPCR; GPR142. GPR142 finns både i mus och i människa och vi valde att studera den i mus. Vi fann att GPR142 är viktig för att bibehålla fungerande β-celler. Aktivering av GPR142 visade sig förbättra insulinfrisättningen samtidigt som produktionen av inflammatoriska och apoptotiska signaler minskade. En apoptotisk signal är en signal till cellen att begå självmord. Vår studie visade att dessa effekter sker genom att aktivering av GPR142 får cellen att producera cAMP, En viktig signalmolekyl i β-cellen.

Sammanfattningsvis visar resultaten i denna avhandling på ett antal proteiner som kan vara attraktiva mål för att utveckla läkemedel som kan förhindra/behandla typ 2 diabetes.

Popular summary

Endocrine signalling systems are an important element in the balanced integration of biological information in an organism. Disruptions in this system may give rise to a variety of diseases, of which metabolic syndrome and diabetes are likely to be the most serious and costly from a societal point of view. Diabetes is a disease where blood sugar is higher than average in both fasting and non-fasting conditions.

The sugar (glucose) is the primary energy source that body cells use and it comes from the food we eat. In order for body cells to absorb the sugar, we need insulin. The insulin is produced and released from the β-cells of the endocrine portion of the pancreas called the islets of Langerhans. A well-regulated release of the insulin from the β-cells has a crucial role in the prevention of metabolic syndrome as well as the constantly elevated blood sugar (hyperglycaemia) and diabetes. Roughly estimated, there are two main types of diabetes, that is type 1 diabetes (lack of insulin-producing β-cells) and type 2 diabetes (T2D), (older age group diabetes, insulin-producing cells remain but with a reduced insulin-releasing capability). The studies presented in my doctoral thesis focus on both investigating the cause of β-cell dysfunction in type 2 diabetes and finding principles that can restore the β-cell function.

As mentioned before a reduced insulin production especially in combination with a pathologically increased insulin resistance leads to hyperglycaemia. It has long been known that hyperglycaemia adversely affects β-cell function. The mechanisms underlying a progressive increase in β-cell dysfunction due to hyperglycaemia which eventually leads to T2D are not fully understood. G-protein coupled receptors (GPCRs) are cell surface receptors that are responsible for carrying the signals from outside to inside the cells. They are one of the most significant and variable drug targets of all the proteins that are expressed on the cell surface.

In the first paper, we have systematically compared the expression of all known human GPCR peptide ligand mRNAs (messenger ribonucleic acid which cause the synthesis of the corresponding protein) with its corresponding gene in mouse pancreatic islets. We created a detailed GPCR/peptide (short chain amino-acids) signal to illustrate the similarities and differences in GPCR peptide ligand expression and signalling pathways. Data presented in this work will allow researchers to focus on GPCR peptide ligands present in human islets but not in

mice, which may lead to the development of new treatments for diabetes by developing peptide ligands adapted for specific GPCRs expressed in human β-cells.

In the second and fourth papers, which are partly related, we have used the information we had developed in our previous GPCR atlas to study signalling systems linked to one of the highly expressed GPCRs in human and mouse β-cells *i.e.* GPR56 (ADGRG1). An important finding in this work was that we discovered that GPR56 agonists may be of interest as a drug candidate for the treatment of type 2 diabetes.

As mentioned above, type 2 diabetes develops after years of pre-diabetes during which high blood sugar (gluco-toxicity) reduces insulin secretion. We report in the third, fourth and fifth papers that ATP-transporting external mitochondrial membrane voltage-dependent anion channel-1 (VDAC1), an important channel in the exchange of molecules and ions between the mitochondria and the cytoplasm, is harmfully increased in β-cells from T2D organ donors and in β-cells from non-diabetic organ donors during prolonged culture under high levels of sugar (glucotoxic conditions). This is caused by a gluco-toxicity-induced transcription program in the β-cell, triggered during years of pre-diabetes and a suboptimal blood sugar control. The VDAC1 overexpression causes its misplacement to the plasma membrane β-cells leading to the outflow of cellular energy (ATP). Subsequent to the loss of ATP, the β-cell cannot function optimally, causing impaired insulin secretion and this can eventually lead to β-cell death. We demonstrate that through the use of specific VDAC1 chemical inhibitors or VDAC1 antibodies, the ATP loss can be prevented and glucose-stimulated insulin release is restored in β-cells from diabetic organ donors. The treatment of a diabetic mouse model with VDAC1 inhibitors prevents the development of hyperglycaemia in these mice. Diabetic mice showed normal glucose tolerance following treatment with VDAC1 the inhibitors.

In the fifth paper, we show the importance of Gpr142, which is expressed in both mouse and human β-cells for the maintenance of β-cell function in rodents. GPR142 activation improves insulin release while also inhibiting the production of inflammatory and apoptotic signals (cellular death). Our study showed that these effects are mediated by the production of cAMP (cyclic adenosine monophosphate), which is an important intracellular signal molecule in the β-cell. In the fourth and fifth paper, we show that there is a reduction of a gene with an important function for the β-cell, which is associated with increased VDAC1 expression. Thus, GPR142 agonists can also be considered as new therapeutic drugs for the treatment of human T2D.

In conclusion, the results in this thesis show that there are some β-cell membrane proteins that can be attractive targets for drug development to prevent, or even treat type 2 diabetes.

ملخص البحث

تعتبر الغدد الصماء عنصراً هاماً في تكامل وتوازن المعلومات البيولوجية في الكائنات الحية وأي اضطرابات في هذا النظام قد تؤدي إلى مجموعة متنوعة من الأمراض ، التي من الغالب أن تتعلق بقابلية الجسم للتمثيل الغذائي ومن ضمنها مرض السكري الأكثر خطورة وتكلفة للفرد والمجتمع . مرض السكري هو مرض مزمن يحدث عندما تكون نسبة السكر في الدم أعلى من المتوسط في كلا الحالتين من الصيام وغير الصيام . يعتبر السكر (الجلوكوز) هو مصدر الطاقة الأساسي الذي تستخدمه خلايا الجسم ويأتي من الطعام الذي نأكله. ومن أجل امتصاص خلايا الجسم للسكر ، نحتاج إلى الإنسولين الذي يتم إنتاجه وإفرازه من خلايا بيتا (β-cells) وهي إحدى الخلايا المكونة لجزر لانجرهانز الموجودة في الجزء المعني بالغدد الصماء في البنكرياس (Pancreatic islets of Langerhans). إن إفراز الأنسولين بشكل جيد من خلايا بيتا له دور حاسم في الوقاية من متلازمة خلل التمثيل الغذائي وكذلك إرتفاع نسبة السكر في الدم (فرط سكر الدم) والسكري. هناك عدة أنواع من مرض السكري ولكن الأكثر شيوعاً هما النوع الأول الذي يصيب الأطفال والشباب في عمر مبكر (Type 1 diabetes) ويحدث بسبب تكوين الجسم لمضادات تصيب الخلايا المنتجة للأنسولين، والنوع الثاني من السكري (Type 2 diabetes) الذي يصيب الفئة العمرية الأكبر سناً (ويتصف بأن الخلايا المنتجة للأنسولين لا تزال موجودة ولكن مع انخفاض القدرة على إفراز الإنسولين أو إنعدام تحسس خلايا الجسم للأنسولين وبالتالي عدم الاستفادة منه. وتركز الدراسات المقدمة في هذه الأطروحة على دراسة أسباب ضعف خلايا بيتا والمؤدية الى مرض السكري من النوع الثاني وإيجاد أفضل السبل التي تمكن من استعادة وظيفة خلايا بيتا أو الحفاظ على انتجيتها لأطول فترة ممكنة لغرض تقليل المضاعفات الناتجة عن المرض . وكما ذكرنا مقدماً إن إنخفاض إنتاج الأنسولين خاصة مع زيادة مقاومة الأنسجة للإنسولين يؤدي إلى فرط سكر الدم . فمن المعروف منذ فترة طويلة أن فرط سكر الدم يؤثر سلباً على وظيفة خلايا بيتا ولكن الآليات الكامنة وراء هذه الزيادة التدريجية في الخلل الوظيفي لخلايا بيتا والمؤديه في النهاية إلى السكري غير مفهومة تمامًا. ولهذا كان اهتمامنا بمستقبلات سطح الخلية (GPCRs) وهي المستقبلات المسؤولة عن حمل الإشارات من خارج الخلايا إلى داخلها والمقترنة بالمحفزات البروتينية الخاصة بكل مستقبل (Peptides). وهي واحدة من أهم الأهداف الدوائية في علم الادوية الحديث لجميع البروتينات التي يتم التعبير عنها على سطح الخلية.

في البحث الأول ، قمنا بمقارنة منهجية بين كل أنواع المستقبلات والمحفزات البروتينية البشرية المعروفة GPCR mRNAs مع مثيلاتها من الفئران في جزر لانجرهانز في البنكرياس (Islets of Langerhans) . لقد توصلنا الى أنشاء أطلس مفصل عن إشارات (GPCR/peptides) لتوضيح التشابهات والاختلافات في مسارات المستقبلات ومسارات المحفزات. ستسمح البيانات المقدمة في هذا الأطلس للباحثين بالتركيز على بروتينات المحفزات الخاصة الموجودة في جزرلانجرهانس البشرية في البنكرياس وليست في في جزر لانجرهانز الموجودة في الفئران، والتي قد تؤدي إلى تطوير علاجات جديدة لمرض السكري عن طريق تطوير روابط محفزات بروتينية مصنعة ومكيّفة لمركبات المستقبلات الخاصة في بالخلايا البشرية.

في البحثين الثاني والرابع، المرتبطان جزئيًا، استخدمنا المعلومات التي طورناها في أطلسنا السابق في دراسة أنظمة مسارات المستقبلات ومسارات المحفزات والمعبر عنها في خلايا لانجرهانز في الإنسان والفئران، وقمنا بدراسة مفصلة لأحد هذه المستقبلات وهو (GPR56\ADGRG1) مع المحفز الخاص به وهو الكولاجين من النوع الثالث والموجود بصورة طبيعية في الفراغ بين الخلايا. من النتائج المهمة في هذا العمل أننا وجدنا أن تحفيز هذه المنبهات بإستعمال الكولاجين من النوع الثالث يؤدي إلى زيادة إفراز الإنسولين من

خلايا بيتا. وفي نفس الوقت وجود آي خلل جيني في هذا المستقبل يؤدي الى نقص افراز الإنسولين حتى مع وجود المحفز الخاص به. علماً إن هذه الدراسة تمت أيضا على جزرلانجرهانس المستخلصة من بنكرياس الأشخاص الواهبين للأعضاء مما يجعل هذه الدراسة موضع اهتمام كمرشح للأدوية لعلاج مرض السكري من النوع الثاني.

كما ذكرنا سابقًا، يتطور مرض السكري من النوع الثاني بعد سنوات من الإصابة الكامنة والمتزايدة والمسماة بالفترة ما قبل السكري (Prediabetes)، حيث يؤدي الارتفاع التدريجي في نسبة السكر في الدم والمسماة سمية الجلوكوز (Gluco-toxicity) إلى تدهور إفراز الإنسولين. في البحثين الثالث والرابع والخامس قمنا بدراسة قنوات خاصة (VDAC-1) والموجودة على الغشاء الخارجي لمولدات الطاقة في الخلية والمسماة الميتوكوندريا (Mitochondria). هذه القنوات تعمل بواسطة اختلاف الجهد الكهربائي وهي مسؤولة عن تبادل الجزيئات والآيونات الموجبة والسالبة ووحدات الطاقة (ATP) بين الميتوكوندريا وسائل السيتوبلازم الخلوي (Cytoplasm). لقد وجدنا زيادة ملحوظة في عدد هذه القنوات في خلايا بيتا المستخلصة من جزر لانجرهانس البشرية للمصابين بالسكري وليس عند مثيلاتها المستخلصة من جزرلانجرهانس البشرية للواهبين الأصحاء مما قد يعزو سبب هذه الزيادة الى السنوات الطويلة من التعرض لسمية الجلوكوز في الخلية. من الجدير بالاهتمام أننا وجدنا أن هذه القنوات تكون أقل عند جزر لانجرهانس البشرية للواهبين المصابين بالسكري والمواظبة على العلاج. يعمل الإفراط في قنوات (VDAC1) الى خلل في تدفق الطاقة الخلوية (ATP) وفقدانها المؤدي الى خلل في عمل المايتوكوندريا والناتج عنه موت خلايا بيتا المسؤولة عن إفراز الإنسولين. ولإثبات هذه النظرية قمنا بإستخدام مثبطات محددة أو أجسام مضادة لقنوات (VDAC1) على خلايا بيتا المستخلصة من جزر لانجرهانس البشرية للمصابين بالسكري ووجدنا تثبيط في حدة فقدان خلايا بيتا للطاقة الخلوية (ATP) مع تحسن ملحوظ في إفراز الإنسولين هذه النتائج شجعت للقيام بمزيد من التجارب لاستخدام مركبات تثبط قنوات (VDAC1) في علاج الفئران المعدلة جينياً لتكون مصابة بداء السكري. أظهرت النتائج تحسن ملحوظ في مستويات سكر الدم وأعراض مرض السكري عند هذه الفئران. تعتبر هذه النتائج واعدة في مجال علاج مرض السكري كما قامت احدى شركات الادوية بتبني مشروع انتاج مركب عقاري مضاد لقنوات(VDAC1) بصدد القيام بدراسات مكثفة في تطبيق العلاج على المتبرعين من مرضى السكري.

في البحث الخامس أظهرنا أيضًا أهمية كبيرة لمستقبل خلوي ثاني موجود في كل من الخلايا البشرية والفئران وهو (GPR142) وقمنا بدراسة اهميته في الحفاظ على وظيفة الخلية بيتا في القوارض. يعمل تنشيط (GPR142) على تحسين إفراز الأنسولين كما أنه يعيق أيضاً إنتاج الإشارات الالتهابية المؤدي تراكمها الى موت خلايا بيتا. كما أستحصلنا على نتائج مشابهة عند تطبيق نفس الدراسة على جزر لانجرهانس البشرية للواهبين المصابين بالسكري (نتائج لم تنشر بعد). أظهرت دراستنا أن هذه التأثيرات يتم توسطها عن طريق إنتاج (cAMP)، وهو جزيء إشارة مهم لخلايا بيتا.

أما في البحثين الرابع والخامس، تظهر أيضاً أهمية حث الجينين (GPR56\ADGRG1) و (GPR142) في القيام بوظيفة المحافظة على خلايا بيتا عن طريق تثبيط قنوات (VDAC1). وبالتالي، يمكن اعتبار المركبات العقارية المنبهه هذين الجينين مهمة باعتبارها نموذج أدوية علاجية جديدة لعلاج مرضى السكري. وبالفعل تم تبني هذه الفكرة من قبل إحدى شركات الادوية وقامت بتكوين مركبات كيميائية محفزة للمستقبل (GPR142) ونقوم حالياً باختبارهاعلى جزرلانجرهانس البشرية للواهبين المصابين بالسكري (نتائج لم تنشر بعد) مقارنة بالواهبين الاصحاء.

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Furthermore, several changes have been made to the original artwork, which include, but are not limited to: changes to colour, merging of shapes, as well as addition and deletion of varying shapes.

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References

1. Goyal, R. and I. Jialal, *Diabetes Mellitus, Type 2*. 2018, StatPearls Publishing LLC.: Treasure Island (FL).
2. DeFronzo, R.A., et al., *Type 2 diabetes mellitus.* Nat Rev Dis Primers, 2015. **1**: p. 15019.
3. ADA, *Diagnosis and classification of diabetes mellitus.* Diabetes Care, 2012. **35 Suppl 1**: p. S64-71.
4. Karamanou, M., et al., *Milestones in the history of diabetes mellitus: The main contributors.* World J Diabetes, 2016. **7**(1): p. 1-7.
5. WHO. *Diabetes*. 2017 [cited 2018; Available from: http://www.who.int/news-room/fact-sheets/detail/diabetes.
6. Skyler, J.S., et al., *Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis.* Diabetes, 2017. **66**(2): p. 241-255.
7. WHO. *Diabetes Programme* 2018 [cited 2018; Available from: http://www.who.int/diabetes/action_online/basics/en/index1.html.
8. Goldenberg, R. and Z. Punthakee, *Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome.* Canadian Journal of Diabetes, 2013. **37**: p. S8-S11.
9. Paschou, S.A., et al., *On type 1 diabetes mellitus pathogenesis.* Endocr Connect, 2018. **7**(1): p. R38-r46.
10. Ellard, S., C. Bellanne-Chantelot, and A.T. Hattersley, *Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young.* Diabetologia, 2008. **51**(4): p. 546-53.
11. De Leon, D.D. and C.A. Stanley, *Permanent Neonatal Diabetes Mellitus*, in *GeneReviews((R))*, M.P. Adam, et al., Editors. 1993, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.: Seattle (WA).
12. Moon, J.H., S.H. Kwak, and H.C. Jang, *Prevention of type 2 diabetes mellitus in women with previous gestational diabetes mellitus.* Korean J Intern Med, 2017. **32**(1): p. 26-41.
13. Genuth, S., et al., *Follow-up report on the diagnosis of diabetes mellitus.* Diabetes Care, 2003. **26**(11): p. 3160-7.
14. Newton, C.A. and P. Raskin, *Diabetic ketoacidosis in type 1 and type 2 diabetes mellitus: clinical and biochemical differences.* Arch Intern Med, 2004. **164**(17): p. 1925-31.
15. ADA, *2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018.* Diabetes Care, 2018. **41**(Suppl 1): p. S13-s27.

16. Abdul-Ghani, M.A., D. Tripathy, and R.A. DeFronzo, *Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose.* Diabetes Care, 2006. **29**(5): p. 1130-9.
17. Sabeti, P.C., et al., *Genome-wide detection and characterization of positive selection in human populations.* Nature, 2007. **449**(7164): p. 913-8.
18. Horikawa, Y., et al., *Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus.* Nat Genet, 2000. **26**(2): p. 163-75.
19. Grant, S.F., et al., *Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes.* Nat Genet, 2006. **38**(3): p. 320-3.
20. da Silva Xavier, G., et al., *TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells.* Diabetes, 2009. **58**(4): p. 894-905.
21. Ferrannini, E., et al., *beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis.* J Clin Endocrinol Metab, 2005. **90**(1): p. 493-500.
22. Ighbariya, A. and R. Weiss, *Insulin Resistance, Prediabetes, Metabolic Syndrome: What Should Every Pediatrician Know?* J Clin Res Pediatr Endocrinol, 2017. **9**(Suppl 2): p. 49-57.
23. DeFronzo, R.A., *Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview.* Diabetologia, 1992. **35**(4): p. 389-97.
24. Del Prato, S., *Loss of early insulin secretion leads to postprandial hyperglycaemia.* Diabetologia, 2003. **46 Suppl 1**: p. M2-8.
25. Wajngot, A., et al., *Quantitative contributions of gluconeogenesis to glucose production during fasting in type 2 diabetes mellitus.* Metabolism, 2001. **50**(1): p. 47-52.
26. DeFronzo, R.A., E. Ferrannini, and D.C. Simonson, *Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake.* Metabolism, 1989. **38**(4): p. 387-95.
27. Pandiri, A.R., *Overview of exocrine pancreatic pathobiology.* Toxicol Pathol, 2014. **42**(1): p. 207-16.
28. Ionescu-Tirgoviste, C., et al., *A 3D map of the islet routes throughout the healthy human pancreas.* Sci Rep, 2015. **5**: p. 14634.
29. Nyman, L.R., et al., *Real-time, multidimensional in vivo imaging used to investigate blood flow in mouse pancreatic islets.* J Clin Invest, 2008. **118**(11): p. 3790-7.
30. Ahren, B., *Autonomic regulation of islet hormone secretion--implications for health and disease.* Diabetologia, 2000. **43**(4): p. 393-410.
31. Brissova, M., et al., *Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy.* J Histochem Cytochem, 2005. **53**(9): p. 1087-97.
32. Arrojo e Drigo, R., et al., *New insights into the architecture of the islet of Langerhans: a focused cross-species assessment.* Diabetologia, 2015. **58**(10): p. 2218-28.

33. Fu, Z., E.R. Gilbert, and D. Liu, *Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes.* Curr Diabetes Rev, 2013. **9**(1): p. 25-53.
34. Bonner-Weir, S., C. Aguayo-Mazzucato, and G.C. Weir, *Dynamic development of the pancreas from birth to adulthood.* Ups J Med Sci, 2016. **121**(2): p. 155-8.
35. Meier, J.J., et al., *Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans.* Diabetes, 2008. **57**(6): p. 1584-94.
36. Chang, S.G., et al., *Role of disulfide bonds in the structure and activity of human insulin.* Mol Cells, 2003. **16**(3): p. 323-30.
37. Hua, Q.X., et al., *A protein caught in a kinetic trap: structures and stabilities of insulin disulfide isomers.* Biochemistry, 2002. **41**(50): p. 14700-15.
38. Koster, J.C., M.A. Permutt, and C.G. Nichols, *Diabetes and insulin secretion: the ATP-sensitive K+ channel (K ATP) connection.* Diabetes, 2005. **54**(11): p. 3065-72.
39. Rorsman, P., et al., *The Cell Physiology of Biphasic Insulin Secretion.* News Physiol Sci, 2000. **15**: p. 72-77.
40. Shibasaki, T., et al., *Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP.* Proc Natl Acad Sci U S A, 2007. **104**(49): p. 19333-8.
41. Furman, B., W.K. Ong, and N.J. Pyne, *Cyclic AMP signaling in pancreatic islets.* Adv Exp Med Biol, 2010. **654**: p. 281-304.
42. Holst, J.J. and J. Gromada, *Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans.* Am J Physiol Endocrinol Metab, 2004. **287**(2): p. E199-206.
43. Hui, H., X. Zhao, and R. Perfetti, *Structure and function studies of glucagon-like peptide-1 (GLP-1): the designing of a novel pharmacological agent for the treatment of diabetes.* Diabetes Metab Res Rev, 2005. **21**(4): p. 313-31.
44. Mathiesen, J.M., L. Vedel, and H. Brauner-Osborne, *cAMP biosensors applied in molecular pharmacological studies of G protein-coupled receptors.* Methods Enzymol, 2013. **522**: p. 191-207.
45. Fridlyand, L.E. and L.H. Philipson, *Pancreatic Beta Cell G-Protein Coupled Receptors and Second Messenger Interactions: A Systems Biology Computational Analysis.* PLoS One, 2016. **11**(5): p. e0152869.
46. Liu, D., et al., *Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP-dependent protein kinase pathway.* Diabetes, 2006. **55**(4): p. 1043-50.
47. Holz, G.G., O.G. Chepurny, and F. Schwede, *Epac-selective cAMP analogs: new tools with which to evaluate the signal transduction properties of cAMP-regulated guanine nucleotide exchange factors.* Cell Signal, 2008. **20**(1): p. 10-20.
48. Kang, G., et al., *cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic beta cells and rat INS-1 cells.* J Physiol, 2006. **573**(Pt 3): p. 595-609.

49. Ashcroft, F.M. and P. Rorsman, *Diabetes mellitus and the beta cell: the last ten years.* Cell, 2012. **148**(6): p. 1160-71.
50. Hales, C.N., et al., *Fetal and infant growth and impaired glucose tolerance at age 64.* Bmj, 1991. **303**(6809): p. 1019-22.
51. Hales, C.N. and D.J. Barker, *Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. 1992.* Int J Epidemiol, 2013. **42**(5): p. 1215-22.
52. Pratley, R.E. and C. Weyer, *The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus.* Diabetologia, 2001. **44**(8): p. 929-45.
53. Dor, Y. and B. Glaser, *beta-cell dedifferentiation and type 2 diabetes.* N Engl J Med, 2013. **368**(6): p. 572-3.
54. Talchai, C., et al., *Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure.* Cell, 2012. **150**(6): p. 1223-34.
55. Nishimura, W., et al., *A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells.* Dev Biol, 2006. **293**(2): p. 526-39.
56. Brereton, M.F., et al., *Reversible changes in pancreatic islet structure and function produced by elevated blood glucose.* Nat Commun, 2014. **5**: p. 4639.
57. Cinti, F., et al., *Evidence of beta-Cell Dedifferentiation in Human Type 2 Diabetes.* J Clin Endocrinol Metab, 2016. **101**(3): p. 1044-54.
58. White, M.G., et al., *Expression of mesenchymal and alpha-cell phenotypic markers in islet beta-cells in recently diagnosed diabetes.* Diabetes Care, 2013. **36**(11): p. 3818-20.
59. Meyer, J., et al., *Acute hyperglycemia alters the ability of the normal beta-cell to sense and respond to glucose.* Am J Physiol Endocrinol Metab, 2002. **282**(4): p. E917-22.
60. Kashyap, S., et al., *A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes.* Diabetes, 2003. **52**(10): p. 2461-74.
61. Chen, C., et al., *Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis.* Mol Metab, 2017. **6**(9): p. 943-957.
62. Henquin, J.C. and J. Rahier, *Pancreatic alpha cell mass in European subjects with type 2 diabetes.* Diabetologia, 2011. **54**(7): p. 1720-5.
63. Butler, A.E., et al., *Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes.* Diabetes, 2003. **52**(1): p. 102-10.
64. Inaishi, J., et al., *Effects of Obesity and Diabetes on alpha- and beta-Cell Mass in Surgically Resected Human Pancreas.* J Clin Endocrinol Metab, 2016. **101**(7): p. 2874-82.
65. Slezak, L.A. and D.K. Andersen, *Pancreatic resection: effects on glucose metabolism.* World J Surg, 2001. **25**(4): p. 452-60.
66. Menge, B.A., et al., *Metabolic consequences of a 50% partial pancreatectomy in humans.* Diabetologia, 2009. **52**(2): p. 306-17.
67. Bradley, D., et al., *Gastric bypass and banding equally improve insulin sensitivity and beta cell function.* J Clin Invest, 2012. **122**(12): p. 4667-74.
68. Bradley, D., F. Magkos, and S. Klein, *Effects of bariatric surgery on glucose homeostasis and type 2 diabetes.* Gastroenterology, 2012. **143**(4): p. 897-912.

69. Menge, B.A., et al., *Partial pancreatectomy in adult humans does not provoke beta-cell regeneration.* Diabetes, 2008. **57**(1): p. 142-9.
70. Del Guerra, S., et al., *Functional and molecular defects of pancreatic islets in human type 2 diabetes.* Diabetes, 2005. **54**(3): p. 727-35.
71. Laybutt, D.R., et al., *Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes.* Diabetologia, 2007. **50**(4): p. 752-63.
72. Marchetti, P., et al., *The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients.* Diabetologia, 2007. **50**(12): p. 2486-94.
73. Mulder, H. and C. Ling, *Mitochondrial dysfunction in pancreatic beta-cells in Type 2 diabetes.* Mol Cell Endocrinol, 2009. **297**(1-2): p. 34-40.
74. Lu, H., et al., *Molecular and metabolic evidence for mitochondrial defects associated with beta-cell dysfunction in a mouse model of type 2 diabetes.* Diabetes, 2010. **59**(2): p. 448-59.
75. Medvedev, A.V., et al., *Regulation of the uncoupling protein-2 gene in INS-1 beta-cells by oleic acid.* J Biol Chem, 2002. **277**(45): p. 42639-44.
76. Maedler, K., et al., *Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets.* Proc Natl Acad Sci U S A, 2004. **101**(21): p. 8138-43.
77. Maedler, K., et al., *Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets.* J Clin Invest, 2002. **110**(6): p. 851-60.
78. Maedler, K., et al., *Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets.* Diabetes, 2001. **50**(8): p. 1683-90.
79. Zalewska, M., M. Siara, and W. Sajewicz, *G protein-coupled receptors: abnormalities in signal transmission, disease states and pharmacotherapy.* Acta Pol Pharm, 2014. **71**(2): p. 229-43.
80. Rosenbaum, D.M., S.G. Rasmussen, and B.K. Kobilka, *The structure and function of G-protein-coupled receptors.* Nature, 2009. **459**(7245): p. 356-63.
81. Millar, R.P. and C.L. Newton, *The Year In G Protein-Coupled Receptor Research.* Molecular Endocrinology, 2010. **24**(1): p. 261-274.
82. Amisten, S., et al., *An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans.* Pharmacol Ther, 2013. **139**(3): p. 359-91.
83. Oldham, W.M. and H.E. Hamm, *Heterotrimeric G protein activation by G-protein-coupled receptors.* Nat Rev Mol Cell Biol, 2008. **9**(1): p. 60-71.
84. Recio, C., et al., *The Role of Metabolite-Sensing G Protein-Coupled Receptors in Inflammation and Metabolic Disease.* Antioxid Redox Signal, 2018.
85. Bittinger, M.A., et al., *Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins.* Curr Biol, 2004. **14**(23): p. 2156-61.
86. Gurevich, V.V. and E.V. Gurevich, *Molecular Mechanisms of GPCR Signaling: A Structural Perspective.* Int J Mol Sci, 2017. **18**(12).
87. Fredriksson, R., et al., *Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives.* FEBS Lett, 2003. **554**(3): p. 381-8.

88. Piao, X., et al., *G protein-coupled receptor-dependent development of human frontal cortex.* Science, 2004. **303**(5666): p. 2033-6.
89. Paavola, K.J., et al., *The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity.* J Biol Chem, 2011. **286**(33): p. 28914-21.
90. Ackerman, S.D., et al., *The adhesion GPCR Gpr56 regulates oligodendrocyte development via interactions with Galpha12/13 and RhoA.* Nat Commun, 2015. **6**: p. 6122.
91. Luo, R., et al., *G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination.* Proc Natl Acad Sci U S A, 2011. **108**(31): p. 12925-30.
92. Van Deijnen, J.H., et al., *Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man.* Cell Tissue Res, 1994. **277**(1): p. 115-21.
93. Olaniru, O.E., et al., *The adhesion receptor GPR56 is activated by extracellular matrix collagen III to improve beta-cell function.* Cell Mol Life Sci, 2018. **75**(21): p. 4007-4019.
94. Li, S., et al., *GPR56 regulates pial basement membrane integrity and cortical lamination.* J Neurosci, 2008. **28**(22): p. 5817-26.
95. Shashidhar, S., et al., *GPR56 is a GPCR that is overexpressed in gliomas and functions in tumor cell adhesion.* Oncogene, 2005. **24**(10): p. 1673-82.
96. Luo, R., et al., *Disease-associated mutations prevent GPR56-collagen III interaction.* PLoS One, 2012. **7**(1): p. e29818.
97. Lin, H.V., et al., *GPR142 Controls Tryptophan-Induced Insulin and Incretin Hormone Secretion to Improve Glucose Metabolism.* PLoS One, 2016. **11**(6): p. e0157298.
98. Mun, H.C., et al., *The Venus Fly Trap domain of the extracellular Ca2+ -sensing receptor is required for L-amino acid sensing.* J Biol Chem, 2004. **279**(50): p. 51739-44.
99. Lin, H.V., et al., *GPR142 prompts glucagon-like Peptide-1 release from islets to improve beta cell function.* Mol Metab, 2018. **11**: p. 205-211.
100. Ueda, Y., et al., *Differential role of GPR142 in tryptophan-mediated enhancement of insulin secretion in obese and lean mice.* PLoS One, 2018. **13**(6): p. e0198762.
101. Engelstoft, M.S., et al., *Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells.* Mol Metab, 2013. **2**(4): p. 376-92.
102. Sommer, C.A. and G. Mostoslavsky, *RNA-Seq analysis of enteroendocrine cells reveals a role for FABP5 in the control of GIP secretion.* Mol Endocrinol, 2014. **28**(11): p. 1855-65.
103. Wang, J., J.J. Carrillo, and H.V. Lin, *GPR142 Agonists Stimulate Glucose-Dependent Insulin Secretion via Gq-Dependent Signaling.* PLoS One, 2016. **11**(4): p. e0154452.
104. Abu-Hamad, S., S. Sivan, and V. Shoshan-Barmatz, *The expression level of the voltage-dependent anion channel controls life and death of the cell.* Proc Natl Acad Sci U S A, 2006. **103**(15): p. 5787-92.

105. Shoshan-Barmatz, V. and D. Gincel, *The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death.* Cell Biochem Biophys, 2003. **39**(3): p. 279-92.
106. Yamamoto, T., et al., *VDAC1, having a shorter N-terminus than VDAC2 but showing the same migration in an SDS-polyacrylamide gel, is the predominant form expressed in mitochondria of various tissues.* J Proteome Res, 2006. **5**(12): p. 3336-44.
107. De Pinto, V., et al., *Characterization of human VDAC isoforms: a peculiar function for VDAC3?* Biochim Biophys Acta, 2010. **1797**(6-7): p. 1268-75.
108. Hinsch, K.D., et al., *Voltage-dependent anion-selective channels VDAC2 and VDAC3 are abundant proteins in bovine outer dense fibers, a cytoskeletal component of the sperm flagellum.* J Biol Chem, 2004. **279**(15): p. 15281-8.
109. Sampson, M.J., et al., *Immotile sperm and infertility in mice lacking mitochondrial voltage-dependent anion channel type 3.* J Biol Chem, 2001. **276**(42): p. 39206-12.
110. Bayrhuber, M., et al., *Structure of the human voltage-dependent anion channel.* Proc Natl Acad Sci U S A, 2008. **105**(40): p. 15370-5.
111. Ujwal, R., et al., *The crystal structure of mouse VDAC1 at 2.3 A resolution reveals mechanistic insights into metabolite gating.* Proc Natl Acad Sci U S A, 2008. **105**(46): p. 17742-7.
112. Gincel, D., S.D. Silberberg, and V. Shoshan-Barmatz, *Modulation of the voltage-dependent anion channel (VDAC) by glutamate.* J Bioenerg Biomembr, 2000. **32**(6): p. 571-83.
113. Thinnes, F.P., et al., *[Identification of human porins. I. Purification of a porin from human B-lymphocytes (Porin 31HL) and the topochemical proof of its expression on the plasmalemma of the progenitor cell].* Biol Chem Hoppe Seyler, 1989. **370**(12): p. 1253-64.
114. Bathori, G., et al., *Extramitochondrial porin: facts and hypotheses.* J Bioenerg Biomembr, 2000. **32**(1): p. 79-89.
115. Buettner, R., et al., *Evidence for secretory pathway localization of a voltage-dependent anion channel isoform.* Proceedings of the National Academy of Sciences of the United States of America, 2000. **97**(7): p. 3201-3206.
116. Zalk, R., et al., *Oligomeric states of the voltage-dependent anion channel and cytochrome c release from mitochondria.* Biochem J, 2005. **386**(Pt 1): p. 73-83.
117. Keinan, N., D. Tyomkin, and V. Shoshan-Barmatz, *Oligomerization of the mitochondrial protein voltage-dependent anion channel is coupled to the induction of apoptosis.* Mol Cell Biol, 2010. **30**(24): p. 5698-709.
118. De Pinto, V., et al., *Determination of the conformation of the human VDAC1 N-terminal peptide, a protein moiety essential for the functional properties of the pore.* Chembiochem, 2007. **8**(7): p. 744-56.
119. Shoshan-Barmatz, V., et al., *VDAC, a multi-functional mitochondrial protein regulating cell life and death.* Mol Aspects Med, 2010. **31**(3): p. 227-85.
120. Tajeddine, N., et al., *Hierarchical involvement of Bak, VDAC1 and Bax in cisplatin-induced cell death.* Oncogene, 2008. **27**(30): p. 4221-32.

121. Chandra, D., et al., *Bax-dependent regulation of Bak by voltage-dependent anion channel 2.* J Biol Chem, 2005. **280**(19): p. 19051-61.
122. Simamura, E., et al., *Furanonaphthoquinones cause apoptosis of cancer cells by inducing the production of reactive oxygen species by the mitochondrial voltage-dependent anion channel.* Cancer Biol Ther, 2006. **5**(11): p. 1523-9.
123. Koren, I., Z. Raviv, and V. Shoshan-Barmatz, *Downregulation of voltage-dependent anion channel-1 expression by RNA interference prevents cancer cell growth in vivo.* Cancer Biol Ther, 2010. **9**(12): p. 1046-52.
124. Arif, T., et al., *Silencing VDAC1 Expression by siRNA Inhibits Cancer Cell Proliferation and Tumor Growth In Vivo.* Mol Ther Nucleic Acids, 2017. **8**: p. 493.
125. Shoshan-Barmatz, V., Y. Krelin, and A. Shteinfer-Kuzmine, *VDAC1 functions in Ca(2+) homeostasis and cell life and death in health and disease.* Cell Calcium, 2018. **69**: p. 81-100.
126. De Stefani, D., et al., *A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter.* Nature, 2011. **476**(7360): p. 336-40.
127. Szabadkai, G., et al., *Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels.* J Cell Biol, 2006. **175**(6): p. 901-11.
128. Madesh, M. and G. Hajnoczky, *VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release.* J Cell Biol, 2001. **155**(6): p. 1003-15.
129. Krols, M., et al., *Mitochondria-associated membranes as hubs for neurodegeneration.* Acta Neuropathol, 2016. **131**(4): p. 505-23.
130. Manczak, M. and P.H. Reddy, *Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease.* Hum Mol Genet, 2012. **21**(23): p. 5131-46.
131. Reddy, P.H., *Is the mitochondrial outermembrane protein VDAC1 therapeutic target for Alzheimer's disease?* Biochim Biophys Acta, 2013. **1832**(1): p. 67-75.
132. Smilansky, A., et al., *The voltage-dependent anion channel 1 mediates amyloid β toxicity and represents a potential target for Alzheimer disease therapy.* Journal of Biological Chemistry, 2015. **290**(52): p. 30670-30683.
133. Ahmed, M., et al., *Mitochondrial proteome analysis reveals altered expression of voltage dependent anion channels in pancreatic beta-cells exposed to high glucose.* Islets, 2010. **2**(5): p. 283-92.
134. Stolar, M., *Glycemic control and complications in type 2 diabetes mellitus.* Am J Med, 2010. **123**(3 Suppl): p. S3-11.
135. Jude, E.B., I. Eleftheriadou, and N. Tentolouris, *Peripheral arterial disease in diabetes--a review.* Diabet Med, 2010. **27**(1): p. 4-14.
136. Mancini, F.R., et al., *Educational level and family structure influence the dietary changes after the diagnosis of type 2 diabetes: evidence from the E3N study.* Nutr Res, 2017. **44**: p. 9-17.
137. McGavock, J., A. Dart, and B. Wicklow, *Lifestyle therapy for the treatment of youth with type 2 diabetes.* Curr Diab Rep, 2015. **15**(1): p. 568.

138. Thrasher, J., *Pharmacologic Management of Type 2 Diabetes Mellitus: Available Therapies.* The American Journal of Cardiology, 2017. **120**(1, Supplement): p. S4-S16.
139. Pawlyk, A.C., et al., *Metformin pharmacogenomics: current status and future directions.* Diabetes, 2014. **63**(8): p. 2590-9.
140. Luizon, M.R., et al., *Genomic Characterization of Metformin Hepatic Response.* PLoS Genet, 2016. **12**(11): p. e1006449.
141. Fullerton, M.D., et al., *Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin.* Nat Med, 2013. **19**(12): p. 1649-54.
142. Viollet, B., et al., *Cellular and molecular mechanisms of metformin: an overview.* Clin Sci (Lond), 2012. **122**(6): p. 253-70.
143. Miller, R.A., et al., *Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP.* Nature, 2013. **494**(7436): p. 256-60.
144. Nyane, N.A., et al., *Metformin-like antidiabetic, cardio-protective and non-glycemic effects of naringenin: Molecular and pharmacological insights.* Eur J Pharmacol, 2017. **803**: p. 103-111.
145. Maleskic, S., et al., *Metformin use associated with protective effects for ocular complications in patients with type 2 diabetes - observational study.* Acta Med Acad, 2017. **46**(2): p. 116-123.
146. Lazarus, B., et al., *Association of Metformin Use With Risk of Lactic Acidosis Across the Range of Kidney Function: A Community-Based Cohort Study.* JAMA Intern Med, 2018. **178**(7): p. 903-910.
147. Lee, E.Y., et al., *Association between Metformin Use and Risk of Lactic Acidosis or Elevated Lactate Concentration in Type 2 Diabetes.* Yonsei Med J, 2017. **58**(2): p. 312-318.
148. Ashcroft, F.M., *Mechanisms of the glycaemic effects of sulfonylureas.* Horm Metab Res, 1996. **28**(9): p. 456-63.
149. Panten, U., M. Schwanstecher, and C. Schwanstecher, *Sulfonylurea receptors and mechanism of sulfonylurea action.* Exp Clin Endocrinol Diabetes, 1996. **104**(1): p. 1-9.
150. Eriksson, J.W., et al., *Sulphonylurea compared to DPP-4 inhibitors in combination with metformin carries increased risk of severe hypoglycemia, cardiovascular events, and all-cause mortality.* Diabetes Res Clin Pract, 2016. **117**: p. 39-47.
151. Shin, M.S., et al., *The duration of sulfonylurea treatment is associated with beta-cell dysfunction in patients with type 2 diabetes mellitus.* Diabetes Technol Ther, 2012. **14**(11): p. 1033-42.
152. Hu, S., et al., *Pancreatic beta-cell K(ATP) channel activity and membrane-binding studies with nateglinide: A comparison with sulfonylureas and repaglinide.* J Pharmacol Exp Ther, 2000. **293**(2): p. 444-52.
153. Hansen, A.M., et al., *Differential interactions of nateglinide and repaglinide on the human beta-cell sulphonylurea receptor 1.* Diabetes, 2002. **51**(9): p. 2789-95.

154. Guardado-Mendoza, R., et al., *The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus.* Arch Med Sci, 2013. **9**(5): p. 936-43.
155. Stein, S.A., E.M. Lamos, and S.N. Davis, *A review of the efficacy and safety of oral antidiabetic drugs.* Expert Opin Drug Saf, 2013. **12**(2): p. 153-75.
156. Rizos, C.V., A. Kei, and M.S. Elisaf, *The current role of thiazolidinediones in diabetes management.* Arch Toxicol, 2016. **90**(8): p. 1861-81.
157. Rizos, C.V., et al., *Pleiotropic effects of thiazolidinediones.* Expert Opin Pharmacother, 2008. **9**(7): p. 1087-108.
158. Rizos, C.V., et al., *How safe is the use of thiazolidinediones in clinical practice?* Expert Opin Drug Saf, 2009. **8**(1): p. 15-32.
159. Yang, T. and S. Soodvilai, *Renal and vascular mechanisms of thiazolidinedione-induced fluid retention.* PPAR Res, 2008. **2008**: p. 943614.
160. Derosa, G., *Efficacy and tolerability of pioglitazone in patients with type 2 diabetes mellitus: comparison with other oral antihyperglycaemic agents.* Drugs, 2010. **70**(15): p. 1945-61.
161. Meier, J.J., *GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus.* Nat Rev Endocrinol, 2012. **8**(12): p. 728-42.
162. Tran, K.L., et al., *Overview of Glucagon-Like Peptide-1 Receptor Agonists for the Treatment of Patients with Type 2 Diabetes.* Am Health Drug Benefits, 2017. **10**(4): p. 178-188.
163. Drab, S.R., *Glucagon-Like Peptide-1 Receptor Agonists for Type 2 Diabetes: A Clinical Update of Safety and Efficacy.* Curr Diabetes Rev, 2016. **12**(4): p. 403-413.
164. Aroda, V.R., et al., *Efficacy of GLP-1 receptor agonists and DPP-4 inhibitors: meta-analysis and systematic review.* Clin Ther, 2012. **34**(6): p. 1247-1258.e22.
165. Chao, E.C., *SGLT-2 Inhibitors: A New Mechanism for Glycemic Control.* Clin Diabetes, 2014. **32**(1): p. 4-11.
166. Scheen, A.J., *Cardiovascular Effects of New Oral Glucose-Lowering Agents: DPP-4 and SGLT-2 Inhibitors.* Circ Res, 2018. **122**(10): p. 1439-59.
167. Monami, M., C. Nardini, and E. Mannucci, *Efficacy and safety of sodium glucose co-transport-2 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials.* Diabetes Obes Metab, 2014. **16**(5): p. 457-66.
168. Stinkens, K. and C. Mathieu, *Sodium-glucose Cotransporter 2 Inhibitors and Ketoacidosis - Clinical Implications in the Treatment of Patients with Type 2 Diabetes.* Eur Endocrinol, 2016. **12**(1): p. 33-34.
169. Chen, H., et al., *Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice.* Cell, 1996. **84**(3): p. 491-5.
170. Lee, G.H., et al., *Abnormal splicing of the leptin receptor in diabetic mice.* Nature, 1996. **379**(6566): p. 632-5.
171. Salehi, A.A. and I. Lundquist, *Islet lysosomal enzyme activities and glucose-induced insulin secretion: effects of mannoheptulose, 2-deoxyglucose and clonidine.* Pharmacology, 1993. **46**(3): p. 155-63.

172. Muhammed, S.J., I. Lundquist, and A. Salehi, *Pancreatic beta-cell dysfunction, expression of iNOS and the effect of phosphodiesterase inhibitors in human pancreatic islets of type 2 diabetes.* Diabetes Obes Metab, 2012. **14**(11): p. 1010-9.
173. Hohmeier, H.E., et al., *Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion.* Diabetes, 2000. **49**(3): p. 424-30.
174. Bompada, P., et al., *Histone acetylation of glucose-induced thioredoxin-interacting protein gene expression in pancreatic islets.* Int J Biochem Cell Biol, 2016. **81**(Pt A): p. 82-91.
175. Bruss, M.L. and A.L. Black, *Enzymatic microdetermination of glycogen.* Anal Biochem, 1978. **84**(1): p. 309-12.
176. Crepaldi, G., et al., *A simplified method of insulin radioimmunoassay. Procedure and preliminary report in normal subjects.* Acta Isot (Padova), 1966. **6**(4): p. 373-91.
177. Panagiotidis, G., et al., *Homologous islet amyloid polypeptide: effects on plasma levels of glucagon, insulin and glucose in the mouse.* Diabetes Res Clin Pract, 1992. **18**(3): p. 167-71.
178. Lequin, R.M., *Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA).* Clin Chem, 2005. **51**(12): p. 2415-8.
179. Wiechelman, K.J., R.D. Braun, and J.D. Fitzpatrick, *Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation.* Anal Biochem, 1988. **175**(1): p. 231-7.
180. Stoscheck, C.M., *Quantitation of protein.* Methods Enzymol, 1990. **182**: p. 50-68.
181. Salvesen, G.S., *Caspases: opening the boxes and interpreting the arrows.* Cell Death Differ, 2002. **9**(1): p. 3-5.
182. Boatright, K.M. and G.S. Salvesen, *Mechanisms of caspase activation.* Curr Opin Cell Biol, 2003. **15**(6): p. 725-31.
183. Rohn, T.T. and E. Head, *Caspase activation in Alzheimer's disease: early to rise and late to bed.* Rev Neurosci, 2008. **19**(6): p. 383-93.
184. Agrawal, N., et al., *RNA interference: biology, mechanism, and applications.* Microbiol Mol Biol Rev, 2003. **67**(4): p. 657-85.
185. Hammond, S.M., et al., *Argonaute2, a link between genetic and biochemical analyses of RNAi.* Science, 2001. **293**(5532): p. 1146-50.
186. Soni, A., et al., *GPRC5B a putative glutamate-receptor candidate is negative modulator of insulin secretion.* Biochem Biophys Res Commun, 2013. **441**(3): p. 643-8.
187. Heid, C.A., et al., *Real time quantitative PCR.* Genome Res, 1996. **6**(10): p. 986-94.
188. Biosystems, A., *Applied Biosystems.* 2018.
189. Schmittgen, T.D. and K.J. Livak, *Analyzing real-time PCR data by the comparative C(T) method.* Nat Protoc, 2008. **3**(6): p. 1101-8.
190. Vandesompele, J., et al., *Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.* Genome Biol, 2002. **3**(7): p. Research0034.

191. Kurien, B.T. and R.H. Scofield, *Western blotting: an introduction.* Methods Mol Biol, 2015. **1312**: p. 17-30.
192. Gething, M.J. and J. Sambrook, *Protein folding in the cell.* Nature, 1992. **355**(6355): p. 33-45.
193. Towbin, H., T. Staehelin, and J. Gordon, *Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications.* Proc Natl Acad Sci U S A, 1979. **76**(9): p. 4350-4.
194. Renart, J., J. Reiser, and G.R. Stark, *Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure.* Proc Natl Acad Sci U S A, 1979. **76**(7): p. 3116-20.
195. Semwogerere, D.a.W., E.R. , *Confocal microscopy* Encyclopedia of Biomaterials and Biomedical Engineering 2005: Tylor & Francis
196. Tantama, M. and G. Yellen, *Imaging changes in the cytosolic ATP-to-ADP ratio.* Methods Enzymol, 2014. **547**: p. 355-71.
197. Tantama, M., et al., *Imaging energy status in live cells with a fluorescent biosensor of the intracellular ATP-to-ADP ratio.* Nat Commun, 2013. **4**: p. 2550.
198. Aram, L., et al., *VDAC1 cysteine residues: topology and function in channel activity and apoptosis.* Biochem J, 2010. **427**(3): p. 445-54.
199. Wiederkehr, A., et al., *Mitochondrial matrix calcium is an activating signal for hormone secretion.* Cell Metab, 2011. **13**(5): p. 601-11.
200. Wagner, B.A., S. Venkataraman, and G.R. Buettner, *The Rate of Oxygen Utilization by Cells.* Free Radic Biol Med, 2011. **51**(3): p. 700-12.
201. Malmgren, S., et al., *Tight coupling between glucose and mitochondrial metabolism in clonal beta-cells is required for robust insulin secretion.* J Biol Chem, 2009. **284**(47): p. 32395-404.
202. Sakmann, B. and E. Neher, *Patch clamp techniques for studying ionic channels in excitable membranes.* Annu Rev Physiol, 1984. **46**: p. 455-72.
203. Gentet, L.J., G.J. Stuart, and J.D. Clements, *Direct measurement of specific membrane capacitance in neurons.* Biophys J, 2000. **79**(1): p. 314-20.
204. Buda, P., et al., *Eukaryotic translation initiation factor 3 subunit e controls intracellular calcium homeostasis by regulation of cav1.2 surface expression.* PLoS One, 2013. **8**(5): p. e64462.
205. Yabe, D. and Y. Seino, *Two incretin hormones GLP-1 and GIP: comparison of their actions in insulin secretion and beta cell preservation.* Prog Biophys Mol Biol, 2011. **107**(2): p. 248-56.
206. Persaud, S.J., *Islet G-protein coupled receptors: therapeutic potential for diabetes.* Curr Opin Pharmacol, 2017. **37**: p. 24-28.
207. Dai, C., et al., *Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets.* Diabetologia, 2012. **55**(3): p. 707-18.
208. Benner, C., et al., *The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression.* BMC Genomics, 2014. **15**: p. 620.
209. Amisten, S., et al., *A comparative analysis of human and mouse islet G-protein coupled receptor expression.* Sci Rep, 2017. **7**: p. 46600.

210. Johnson, K.H., et al., *Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic beta cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques.* Am J Pathol, 1988. **130**(1): p. 1-8.
211. Khan, D., et al., *Influence of neuropeptide Y and pancreatic polypeptide on islet function and beta-cell survival.* Biochim Biophys Acta Gen Subj, 2017. **1861**(4): p. 749-758.
212. Wiederkehr, A. and C.B. Wollheim, *Mitochondrial signals drive insulin secretion in the pancreatic β-cell.* Molecular and Cellular Endocrinology, 2012. **353**(1-2): p. 128-137.
213. Doliba, N.M., et al., *Glucokinase activation repairs defective bioenergetics of islets of Langerhans isolated from type 2 diabetics.* American Journal of Physiology - Endocrinology and Metabolism, 2012. **302**(1): p. E87-E102.
214. Fernandez-Echevarria, C., et al., *Aβ promotes VDAC1 channel dephosphorylation in neuronal lipid rafts. Relevance to the mechanisms of neurotoxicity in Alzheimer's disease.* Neuroscience, 2014. **278**: p. 354-366.
215. De Pinto, V., et al., *Voltage-dependent anion-selective channel (VDAC) in the plasma membrane.* FEBS Lett, 2010. **584**(9): p. 1793-9.
216. Shalev, A., *Minireview: Thioredoxin-interacting protein: Regulation and function in the pancreatic β-cell.* Molecular Endocrinology, 2014. **28**(8): p. 1211-1220.
217. Ben-Hail, D., et al., *Novel Compounds Targeting the Mitochondrial Protein VDAC1 Inhibit Apoptosis and Protect against Mitochondrial Dysfunction.* J Biol Chem, 2016. **291**(48): p. 24986-25003.
218. Akanda, N., et al., *Voltage-dependent anion channels (VDAC) in the plasma membrane play a critical role in apoptosis in differentiated hippocampal neurons but not in neural stem cells.* Cell Cycle, 2008. **7**(20): p. 3225-3234.
219. Aram, L., et al., *VDAC1 cysteine residues: Topology and function in channel activity and apoptosis.* Biochemical Journal, 2010. **427**(3): p. 445-454.
220. Cao, K., et al., *Hydroxytyrosol prevents diet-induced metabolic syndrome and attenuates mitochondrial abnormalities in obese mice.* Free Radic Biol Med, 2014. **67**: p. 396-407.
221. Duner, P., et al., *Adhesion G Protein-Coupled Receptor G1 (ADGRG1/GPR56) and Pancreatic beta-Cell Function.* J Clin Endocrinol Metab, 2016. **101**(12): p. 4637-4645.
222. Little, K.D., M.E. Hemler, and C.S. Stipp, *Dynamic regulation of a GPCR-tetraspanin-G protein complex on intact cells: central role of CD81 in facilitating GPR56-Galpha q/11 association.* Mol Biol Cell, 2004. **15**(5): p. 2375-87.
223. Poungvarin, N., et al., *Carbohydrate response element-binding protein (ChREBP) plays a pivotal role in beta cell glucotoxicity.* Diabetologia, 2012. **55**(6): p. 1783-96.
224. Shalev, A., *Minireview: Thioredoxin-interacting protein: regulation and function in the pancreatic beta-cell.* Mol Endocrinol, 2014. **28**(8): p. 1211-20.
225. Singh, S.P., et al., *Different developmental histories of beta-cells generate functional and proliferative heterogeneity during islet growth.* Nat Commun, 2017. **8**(1): p. 664.

226. Toda, N., et al., *Potent and Orally Bioavailable GPR142 Agonists as Novel Insulin Secretagogues for the Treatment of Type 2 Diabetes.* ACS Med Chem Lett, 2013. **4**(8): p. 790-4.
227. Kumar, R., et al., *Insulinotropic and antidiabetic effects of 17beta-estradiol and the GPR30 agonist G-1 on human pancreatic islets.* Endocrinology, 2011. **152**(7): p. 2568-79.

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Prevention of Pancreatic β-Cell Failure in Type 2 Diabetes

Israa Mohammed Al-Amily is a medical doctor who obtained her MSc in Medical Genetics from the University of Glasgow. The focus of her doctoral thesis is to investigate the role of the mitochondrial voltage dependent anion channels and the modulatory effects of two novel GPCRs in pancreatic beta-Cells, aiming at targeting them as a therapeutic approach for prevention and treatment of T2D.

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