Chapter 6

The Impact of Inflammation on Pancreatic β-Cell Metabolism, Function and Failure in T1DM and T2DM: Commonalities and Differences

Philip Newsholme, Kevin Keane,
Paulo I Homem de Bittencourt Jr. and
Mauricio Krause

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55349

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronically progressive autoimmune disease that affects approximately 1% of the population in the developed world. This adverse immune response is induced and promoted by the interaction of both genetic and environmental factors. In contrast, in type 2 diabetes mellitus (T2DM), insulin-resistance coupled with reduced insulin output appears to be the major cause of hyperglycaemia (affecting approximately 6% of the population). Although the aetiology of diabetes may differ from T1DM to T2DM, a common feature associated with both types is the failure of pancreatic β-cells in the islets of Langerhans, thus causing a reduction in insulin secretion, cell mass and ultimately apoptotic death. However, the impact and time-course of pancreatic β-cell death, which may appear very different in T1 and T2DM, may be related through common molecular mechanisms.

Glucose-stimulated insulin secretion (GSIS) is central to the physiological control of metabolic fuel homeostasis, and its impairment is a hallmark of pancreatic β-cell failure in T2DM. β-Cells are often referred to as "fuel sensors" as they continually monitor and respond to dietary nutrients, under the modulation of additional neuro-hormonal and immunological signals, in order to secrete insulin to best meet the needs of the organism. Therefore, β-cell dysfunction and death in diabetes leads to hyperglycaemia and its complications. An intriguing characteristic of the pancreatic β-cells is their similarity to immune cells: 1) they can release cytokines; 2) they strongly respond to cytokines from other cells and tissues; 3) their function is dependent on the production of reactive oxygen (ROS) and nitrogen species (RNS); 4) they express high

levels of pro-inflammatory proteins such as nuclear transcription factor κB (NFκB), inducible nitric oxide synthase (iNOS), NADPH oxidase (NOX), Toll-like receptors (TLR) and other proteins in response to immune signals, but also to metabolic challenge. However and in contrast to professional immunoinflammatory cells, such as macrophages or neutrophils, the β-cell is fragile when subjected to immune attack and is highly vulnerable to oxidative stress.

In this chapter, we intend to review the mechanisms of insulin secretion in response to a wide variety of metabolic stimuli, the 'immune-like' characteristics of the pancreatic β-cells with respect to metabolism, secretion and cell defence, the similarities between β-cell failure/death in T1DM and T2DM and finally, to suggest novel targets for the treatment of diabetes.

2. Regulation of β-cell function and insulin secretion

Control of energy metabolism is essential in maintaining cellular homeostasis in all animals across the metazoan (all animals with differentiated tissues). Insulin and glucagon are hormones produced by vertebrate organisms to regulate glycaemic homeostasis. In addition, insulin-like and glucagon-like peptide genes have been detected in invertebrate organisms including, insects, molluscs and nematodes, thus inferring a similar metabolic control that is conserved among most species [1,2]. However, in the case of vertebrates, insulin and glucagon are produced by cells located in the islets of Langerhans of the animal pancreas. Under normal physiological conditions, blood glucose concentration is maintained within narrow limits by an alternate release of these powerful proteins, regardless of nutrient intake or expenditure (*e.g.* exercise). There are four main cell types that contribute to the regulation of this pancreatic function and they include, α-cells, β-cells, δ-cells and pancreatic peptide (PP)-cells [3]. The role of α-cells is to synthesise and secrete glucagon in response to low extracellular glucose concentrations, thus replenishing the plasma carbohydrate level [3]. δ-Cells secrete somatostatin that has an inhibitory effect on insulin and glucagon release, while PP-cells secrete pancreatic peptide whose physiological function has not been fully elucidated [3]. Conversely, the function of β-cells has been extensively studied and they are responsible for the biosynthesis and release of insulin in response to elevated plasma glucose, amino acid and saturated fatty acid levels [3]. These cells represent the most abundant cell type in pancreatic islets and are the primary source of dysfunction in DM.

β-Cell responsiveness and subsequent insulin secretion is subject to a plethora of cellular regulatory mechanisms. Insulin biosynthesis and secretion is a highly controlled system that has many influencing extracellular and intracellular factors including, glucose, fatty acids, amino acids, nucleotides, calcium/potassium electrochemical gradient, metabolic coupling factors (MCFs), and level of ROS and RNS. Furthermore, the fact that cellular insulin secretion is achieved by the physical release of vesicles or granules containing the protein, suggests that the process acquires a greater degree of complexity and control, and is subject to vesicle manufacture, recruitment and finally plasma membrane docking.

Glucose-Stimulated Insulin Secretion (GSIS) is fundamental to insulin exocytosis as glucose is the most potent insulin secretagogue [4]. In an environment of excess extracellular glucose,

http://dx.doi.org/10.5772/55349

β-cell plasma membrane transporter proteins GLUT1 and GLUT2, actively transport free glucose molecules inside the cell where glycolysis can be initiated to create the nucleotide ATP (Fig. 1). Consequently, intracellular metabolism of glucose by glycolysis, and further metabolism of pyruvate via the downstream tricarboxylic acid (TCA) cycle, leads to elevated NADH, $FADH_2$ and ultimately ATP levels [4]. The increased intracellular ATP:ADP ratio closes membrane-bound ATP-sensitive K^+ channels, resulting in plasma membrane depolarisation and a subsequent opening of membrane-bound voltage activated Ca^{2+} channels. A rapid influx of calcium ions is promoted, causing the exocytosis of insulin through fusion of the insulin containing vesicles with the plasma membrane via VAMP (vesicle-associated membrane protein) and SNARE (soluble NH2-ethylmaleimide-sensitive fusion protein attachment protein receptor) association [5]. This specific process of insulin secretion is known as K_{ATP}-dependent GSIS, and since ATP generation is critical, the metabolic control points of glycolysis, the TCA cycle and oxidative phosphorylation (*i.e.* activity of metabolic enzymes such as hexokinase, phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase, pyruvate carboxylase, glutamate dehydrogenase and mitochondrial redox-shuttles) have a significant impact on regulation of insulin release.

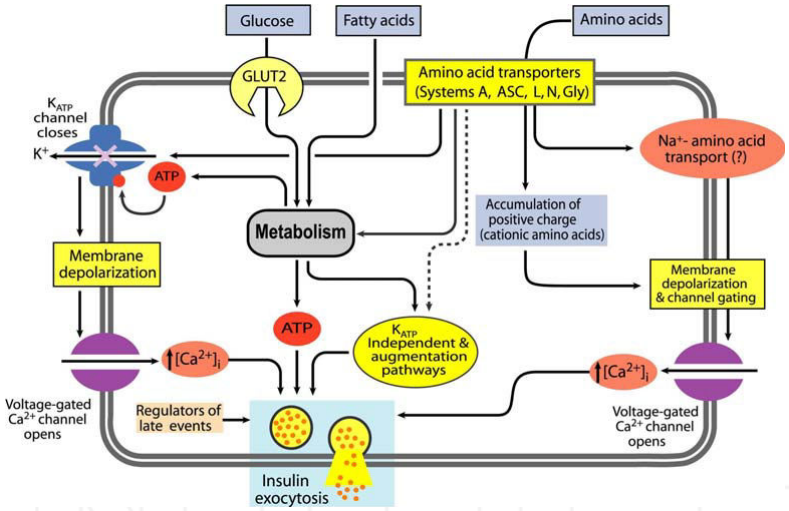

Figure 1. Mechanisms of nutrient and amino acid stimulated insulin secretion. Glucose metabolism is essential for stimulation of insulin secretion. The mechanisms by which amino acids enhance insulin secretion are understood to primarily rely on (a) direct depolarization of the plasma membrane (e.g. cationic amino acid, L-arginine); (b) metabolism (e.g. alanine, glutamine, leucine); and (c) co-transport with Na^+ and cell membrane depolarization (e.g. alanine). Notably, rapid partial oxidation may also initially increase both the cellular content of ATP (impacting on K^+ATP channel closure prompting membrane depolarization) and other stimulus secretion coupling factors. In the absence of glucose, fatty acids may be metabolised to generate ATP and maintain basal levels of insulin secretion. Adapted from [3].

However, there also remains the possibility that K_{ATP}-independent GSIS can occur in the β-cell, although the exact methodology is still not fully understood. K_{ATP}-independent GSIS has been illustrated in studies utilising diazoxide to maintain K^+ channels in the open position [6]

and in mice with disrupted/deleted K^+ channels [7, 8]. GSIS was subsequently shown to be possible in a K_{ATP}-independent manner and it is believed that these two co-ordinate mechanisms of insulin secretion (*i.e.* K_{ATP}-dependent & K_{ATP}-independent GSIS), are responsible for the bi-phasic insulin response in animals. It is thought that the initial rise in insulin secretion is K_{ATP}-dependent, while the second phase is mediated through K_{ATP}-independent interactions dependent on mitochondrial activity [4,9].

Mitochondrial, lipid and amino acid metabolism plays a significant role in regulation of insulin secretion and GSIS. Lipid and amino acid metabolites can generate, or can directly become MCFs that enhance or inhibit GSIS. While individual amino acids alone at physiological concentrations do not enhance GSIS, some specific amino acids at higher concentrations, or in combination with others, can cause increments in GSIS [10]. Arginine, alanine, leucine and glutamine can increase GSIS, while homocysteine and cysteine at elevated concentration can inhibit GSIS [10]. The effect of amino acids is also dependent on whether β-cells are exposed acutely or chronically, as chronic exposure may influence the expression of genes involved in the control of insulin secretion [10,11]. In addition, another nutrient source, fatty acids, can also regulate GSIS in both a positive or negative manner depending on the level of saturation, carbon chain length, and whether exposure is under acute or chronic conditions. Saturated fatty acids like palmitic and stearic acid are known to chronically decrease GSIS *in vitro*, but palmitic acid can acutely enhance GSIS [12-14]. Conversely, chronic exposure to monounsaturated oleic acid and polyunsaturated arachidonic acid can increase insulin production in β-cells [13,15]. Fatty acids can amplify β-cell GSIS, and it is likely that they elevate insulin levels by causing changes in calcium influx and proteins associated with ion channel activity [16]. Mitochondrial metabolism of amino and fatty acid is at the hub of the reported effects on insulin secretion and GSIS, mainly because TCA-mediated metabolism of both leads to increased ATP production and protein biosynthesis, which is a prerequisite for insulin secretion (Fig. 1). The intricacies of mitochondrial-mediated metabolism of amino and fatty acids will be discussed below.

3. Pancreatic β-cell metabolism and influencing factors

Pancreatic β-cells are unique and can be distinguished from other cell types by their metabolic profile. Several key characteristics of β-cells include the ability to utilise glucose in the physiological range of 2-20mmol/L, express low levels of lactate dehydrogenase (LDH) and plasma membrane monocarboxylate pyruvate/lactate transporter, have a corresponding high activity of glycerol-3-phosphate and malate/aspartate redox shuttles, and finally possess an elevated level of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) activity, ensuring that both oxidative and anaplerotic metabolism of glucose and pyruvate can occur preferentially in the near absence of lactate generation (Fig. 2) (further details can be found in [4,10,11,17-21]). These adaptions are designed to specifically accelerate oxidative phosphorylation and TCA activity as a means to increase ATP output and consequently insulin exocytosis.

http://dx.doi.org/10.5772/55349

Figure 2. Schematic diagram representing the metabolism of selected amino acids, highlighting related metabolic stimulus-secretion coupling factors involved in insulin release. The pathway of glutamine metabolism via glutaminase, GDH, and entry into the TCA cycle (glutaminolysis) is shown along with key points of amino acid interaction with glutamine and glucose metabolism. KG, -ketoglutarate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AT, aminotransferase; BCKDH, branched-chain-keto-acid dehydrogenase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; KIC, ketoisocaproic acid. Adapted from [21].

Pancreatic β-cells regenerate NAD^+ for glycolysis primarily through high expression of mitochondrial NADH shuttles like glycerol-3-phosphate and the malate/aspartate shuttle (Fig. 3), for specific details refer to [11,22]. Briefly, the glycerol-3-phosphate shuttle consists of cytosolic and mitochondrial glycerol-3-phosphate dehydrogenase that operate in unison to convert dihydroxyacetone phosphate to glycerol-3-phosphate and NAD^+, with a subsequent generation of $FADH_2$ from NAD^+ [4]. In contrast, the malate/aspartate shuttle is the main shuttle responsible for transferring glycolytic reducing equivalents to the mitochondria in the β-cell [11]. Here, cytosolic malate dehydrogenase reduces oxaloacetate to malate and NAD^+, with a subsequent generation of NADH inside the mitochondria. Using an amino group provided by glutamate, mitochondrial oxaloacetate can be converted back to aspartate maintaining this cyclic event. The malate/aspartate shuttle is dominantly expressed in β-cells, eloquently linking glycolysis to mitochondrial & amino acid metabolism.

As alluded to previously, amino acid metabolism is essential for nutrient- and glucose-stimulated insulin secretion, and the effects of several amino acids have been reviewed extensively [3,10, 11]. To summarise these findings briefly, both arginine and alanine have been shown to promote insulin release through changes in electrogenic transport, progressing to activation of Ca^{2+} ion channels [10,23,24]. It has also been demonstrated that they enhance glutamate production and consequently may play a role in malate/aspartate shuttle-mediated generation of NADH, and/or in glutathione synthesis and antioxidant defence [25]. Therefore, both arginine and alanine may

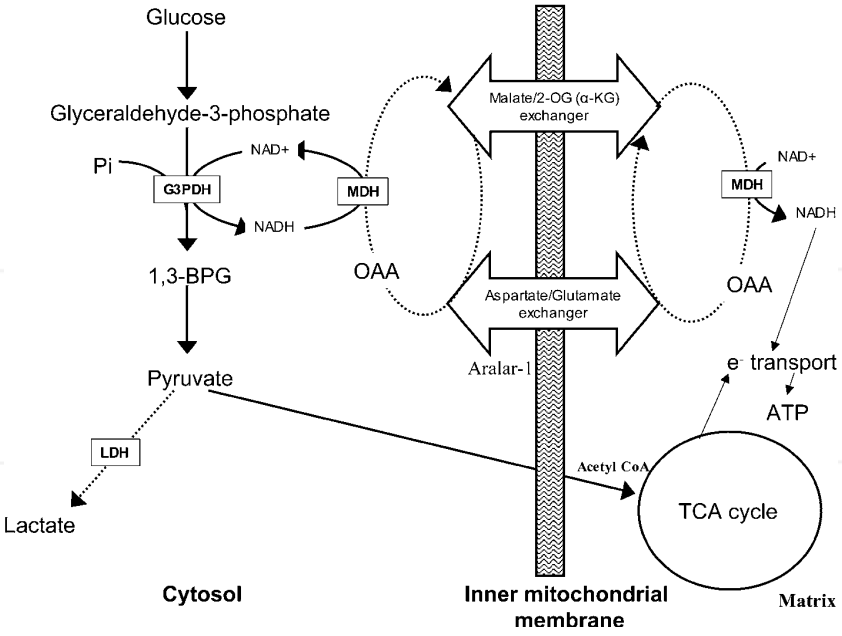

Figure 3. The malate–aspartate shuttle is the principal mechanism for the movement of reducing equivalents in the form of NADH from the cytoplasm to the mitochondrion in β-cells. Cytoplasmic malate dehydrogenase (MDH) reduces oxaloacetate (OAA) to malate while oxidizing NADH to NAD+. Malate then enters the mitochondrion where the reverse reaction is performed by mitochondrial malate dehydrogenase. Movement of mitochondrial oxaloacetate to the cytoplasm to maintain this cycle is achieved by transamination to aspartate with the amino group being donated by glutamate. The 2-oxoglutarate (*α*-ketoglutarate) generated leaves the mitochondrion for the cytoplasm. Adapted from [11].

protect β-cells from oxidative insult in addition to promoting insulin secretion. However, prolonged exposure of β-cells to alanine results in decreased alanine-induced insulin secretion, while reaction of arginine with inducible nitric oxide synthase (iNOS) can promote nitric oxide (NO) production [10,19]. NO is an important signalling molecule, which is essential for β-cell glucose uptake at low levels, but at high concentration may be toxic [26]. Interaction of NO with superoxide (O^-) can also lead to the formation of peroxynitrite ($ONOO^-$), a damaging free radical that can disrupt mitochondrial function [27]. In fact, $ONOO^-$, which is in equilibrium with its conjugate peroxynitrous acid (ONOOH, $pK_a \approx 6.8$) [28], is a highly reactive oxidant species produced by the combination of the oxygen free radical O_2^- and NO [29] and has been demonstrated to be a more potent oxidant and cytotoxic mediator than NO or O_2^- individually, in a variety of inflammatory conditions [30]. $ONOO^-$ is extremely cytotoxic to rat and human islet cells *in vitro* [31] and its *in vivo* formation has been reported in pancreatic islets where it has been associated with β-cell destruction and development of T1DM in NOD mice [32].

High levels of homocysteine and cysteine have also been shown to elicit a negative effect on β-cell function. In obese hyperinsulinaemic T2DM patients, homocysteine levels are increased, while they are increased in T1DM patients, but only following disease-related complications

http://dx.doi.org/10.5772/55349

such as diabetic nephropathy [11,33]. It has been suggested that homocysteine can decrease GSIS in rat pancreatic β-cells [34], although the inhibitory mechanism is still not fully understood. It may decrease insulin secretion by altering enzyme and/or protein activity, or by causing oxidative stress [35,36]. In addition, homocysteine can be converted to asymmetric dimethylarginine, which is inhibitor of neuronal NOS and can also inhibit iNOS to a lesser extent and therefore may reduce NO production, which is important for β-cell insulin secretion and function [10,37]. In contrast, cysteine has been shown to increase β-cell GSIS at low concentrations [38] and is essential for antioxidant defence and glutathione synthesis, along with glycine and glutamate. Cysteine supplementation was found to protect β-cells from hydrogen peroxide (H_2O_2)-induced cell death and prevented glucotoxicity in mouse β-cells [39,40]. However, at elevated concentrations, it impaired GSIS through excessive hydrogen sulphide (H_2S) formation [41].

Glutamine is required for β-cell metabolism and function, and is consumed at rapid rates [10]. Glutamine supplementation does not induce insulin release [10], but co-treatment with leucine significantly enhances GSIS via activation of glutamate dehydrogenase (GDH), allowing entry of glutamine into the TCA cycle (Fig. 2) [42]. It has been suggested that glutamine alone does not induce insulin secretion because it is not oxidised during its metabolism. Instead, metabolism of glutamine may yield aspartate and GABA (γ-aminobutyric acid), a potent inhibitor of glucagon secretion (Fig. 2) [3]. However, using NMR studies, we found that the major products of glutamine metabolism were aspartate and glutamate. Here, glutamate entered the γ-glutamyl cycle and increased the synthesis of the antioxidant, glutathione [43]. Formation of glutamate from glutamine also has important implications in activation of the aspartate/glutamate shuttles and in ATP production from the TCA cycle, via glutamate metabolism to α-ketoglutarate. Consequently, glutamine may function to enhance ATP production and insulin release by changes in down-stream metabolism, most notably via glutamine-derived glutamate. Alternatively, glutamate can be transported externally from the cell and into the surrounding matrix, which may cause glutamate receptor activation and desensitisation if the rate of release is over extended periods [44]. Since glucagon secretion from pancreatic α-cells is sensitive to glutamate exposure, its release may represent a novel paracrine control mechanism for modulation of blood carbohydrate levels [44]. Some groups have reported that total intracellular glutamate levels increased in response to glucose, while others reported no significant change [25,45,46]. Recently, it has been suggested that glutamate is transported into insulin-containing vesicles, thereby promoting Ca^{2+}-dependent insulin secretion [47]. However, the role of glutamate in mediating insulin secretion remains hotly debated.

Taken together, this evidence suggests that a variety of amino acids may contribute significantly to regulation of pancreatic β-cell insulin secretion. However, other β-cell metabolic processes are important to insulin secretion and must be considered. These include four key metabolic shunts that divert glucose from being utilised by TCA cycle (*i.e.*, aldose reductase, pentose-phosphate, glycogen synthesis and hexosamine pathways; please, see Fig. 4) as well as down-stream glycolytic enzymes such as PC and PDH, and also enzymes involved in fatty acid metabolism like acetyl CoA carboxylase (ACC) and fatty acid synthase.

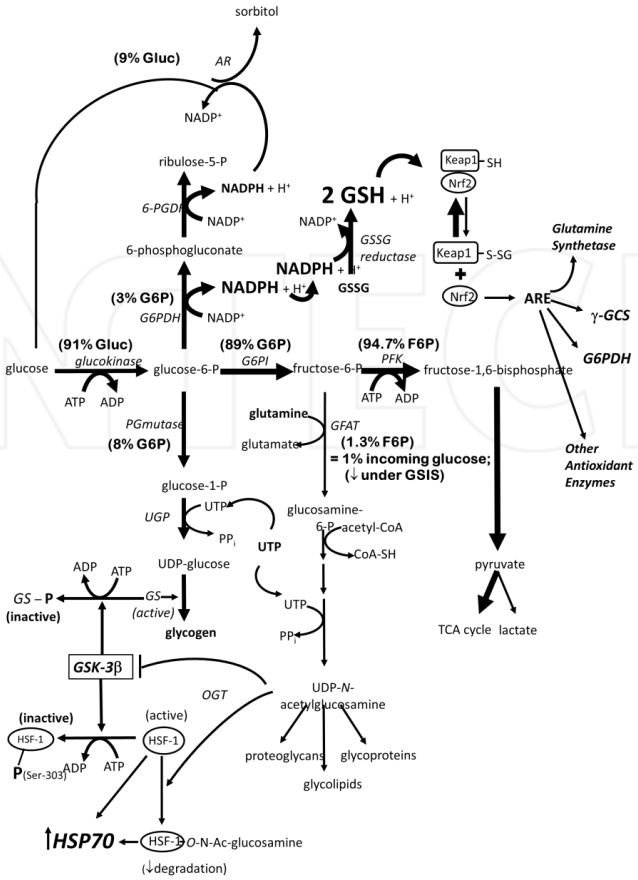

Figure 4. Flux balance analysis of glucose utilisation in β-cells. The fluxes through the biochemical pathways shown here were calculated by using Michaelis-Menten function, intracellular metabolite concentrations estimated from different works. Percentages in parentheses refer to the proportional amount of the metabolite consumed through that step. AR, aldose reductase; ARE, antioxidant response (ARE) elements in the promoter regions of target genes; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; G6PI, glucose-6-phosphate isomerase (a.k. as phosphoglucoisomerase); γ-GCS, glutamate cysteine ligase, a.k. as γ-glutamylcysteine synthetase; GFAT, glutamine:fructose-6-phosphate amidotransferase a.k. as GFPT, for glutamine-fructose-6-phosphate transaminase; Gluc, glucose; GS, glycogen synthase; GSIS, glucose-stimulated insulin secretion; GSK-3β, glycogen synthase kinase-3β; HSF-1, heat shock transcription factor-1; HSP70, the 70-kDa family of heat shock proteins (includes both hsp72, encoded by the HSPA1A gene, and hsp73, a.k. as hsc70, encoded by the HSPA8 gene); Keap1, Kelch-like ECH-associated protein 1; Nrf2, Nuclear factor erythroid 2-related transcription factor 2; OGT, *O*-*N*-acetylglucosamine transferase, a.k. as UDP-*N*-acetyl-D-glucosamine:protein-*O*-β-*N*-acetyl-D-glucosaminyl transferase and uridine diphospho-*N*-acetylglucosamine:polypeptide β-*N*-acetylglucosaminyl transferase; PFK, phosphofructokinase; PGmutase, phosphoglucomutase; UGP, UDP-glucose pyrophosphorylase.

Highlighting the peculiarities of β-cell metabolism in a coordinated effort to increase the activity of a number of metabolic pathways in response to glucose, Huang & Joseph (2012) have shown, by using metabolomic analysis, during GSIS in clonal β-cells, a conspicuous

http://dx.doi.org/10.5772/55349

accumulation of pyruvate, succinate, fumarate, malate, α-ketoglutarate, dihydroxyacetone phosphate (DHAP), (iso)citrate, palmitate, glucose-6-phosphate and 6-phosphogluconate whereas aspartate was consumed in response to glucose [48]. Here, the authors have clearly demonstrated that under glucose stimulus, β-cells strongly enhance metabolic flux towards glycolysis and TCA cycle. Indeed, there is a very delicate poise to coordinately regulate the flux of glucose towards the formation of NADPH (through the pentose-phosphate shunt) avoiding excessive formation of sorbitol (via the polyol-aldose reductase shunt) which would empty glycolytic flux (Fig. 4). It has long been recognised, for instance, that overexpression of the aldose reductase gene is able to induce apoptosis in pancreatic β-cells by causing a redox imbalance [49] while, on the contrary, pharmacological blockage of aldose reductase may impair GSIS, thus suggesting that the conversion of free intracellular glucose to sorbitol in the β-cell is an essential step in the glucose-induced release mechanism (Fig.4) [50].

Although the physiological significance is still under debate, glucose 6-phosphate may also be targeted towards glycogen synthesis in pancreatic islets, which is enhanced during GSIS and impaired in STZ-diabetic rats (Fig. 4) [51,52]. Finally, glucose may be deviated from ultimate metabolism through further glycolytic steps via the reaction of fructose-6-phosphate with glutamine through the hexosamine biochemical pathway (HBP) (Fig. 4). Increased fluxes through HBP may, on the one hand, block glycogen synthase kinase-3β (GSK-3β), thus liberating glycogen synthesis by glycogen synthase and, on the other, may reduce heat shock factor-1 (HSF-1) degradation thus allowing enhanced expression of the 70-kDa heat shock protein (HSP70), which is cytoprotective to β-cells (Fig. 4) [53,54]. Over-enhanced flux through HBP is an inducer of endoplasmic reticulum (ER) stress, while being associated with insulin-resistance [55].

PC and PDH are both highly expressed in β-cells and allow conversion of pyruvate to oxaloacetate (PC) and acetyl-CoA (PDH), with subsequent entry into the TCA cycle [4]. Interestingly, siRNA inhibition of PC reduces cell proliferation and GSIS in insulinoma cells and rat islets, while overexpression in rat islets could enhance GSIS and cell proliferation [56, 57]. The role of PDH is less understood and it is thought to support PC activity by providing acetyl-CoA for citrate production. Both enzymes are important regulators of the pyruvate/malate and pyruvate/citrate shuttles. Common to each pathway is the conversion of glycolytic-derived pyruvate to oxaloacetate by PC, as described above. In the case of the pyruvate/malate shuttle, oxaloacetate is then converted to malate and translocated to the cytosol, where malic enzyme1 (ME1) converts malate back to pyruvate along with generation of NADPH. Pyruvate can re-enter the mitochondria to repeat the cycle with further generation of NADPH [4]. However, for the pyruvate/citrate shuttle, PC-mediated oxaloacetate leads to condensation with acetyl CoA (possibly generated by PDH), and the subsequent formation of citrate. Translocation of citrate to the cytosol results in oxaloacetate and acetyl CoA regeneration from citrate by ATP-citrate lyase (ACL). Oxaloacetate re-enters the pyruvate/malate cycle with generation of NADPH as outlined previously, while acetyl CoA is carboxylated to malonyl CoA by acetyl CoA carboxylase (ACC). Malonyl CoA is then converted to long chain acyl CoA by fatty acid synthase leading to fatty acid production. Additionally, malonyl CoA can also inhibit carnitine palmitoyl transferase 1 (CPT-1), which in a low glucose state, transports fatty

acids into the mitochondria to generate ATP by oxidation [4,10]. However, in high glucose situations, inhibition of CPT-1 leads to fatty acid accumulation in the cytosol and this accumulation may increase insulin exocytosis by augmenting calcium influx and ion channel proteins [10,16]. Interestingly, formation of malonyl CoA from acetyl CoA by ACC is positively regulated by the glutamine-sensitive protein phosphatase type 2A (PP2A), while it is negatively regulated by the amino acid-sensitive AMP-activated kinase (AMPK) [11,58,59]. These concepts again fully illustrate the inherent relationship between β-cell metabolism of glucose, amino acids and lipids with insulin exocytosis [11,58,59].

AMPK is crucial in lipid metabolism control and can chronically regulate β-cell function by altering the expression of vital transcription factors that govern lipogenic and glycolytic enzymes [10]. Chronic exposure of β-cells to high circulatory lipid levels, as occurs in T2DM, can inhibit glucose oxidation and result in a decreased ATP/AMP ratio along with a subsequent activation of AMPK, which inhibits fatty acid synthesis, while enhancing fatty acid oxidation, and impairing GSIS [10]. The exact metabolic mechanisms of how lipids can augment GSIS are still not fully understood but are believed to involve modulation of Ca^{2+} mobilisation via interaction with G-protein coupled receptors [60]. Recent evidence has shown that these G-protein coupled receptors are highly expressed in β-cells and correlated with insulinogenic index [10,61]. It has also been demonstrated that interaction of omega-3 fatty acids and the GPR120 receptor, plays an instrumental role in mediating insulin-sensitisation and anti-inflammatory effects in obese mice models [62].

AMPK also occupies a central position in metabolic regulation in order to avoid inflammatory dysregulation. Accordingly, in different cell types, AMPK phosphorylates and inhibit glutamine:fructose-6-phosphate amidotransferase-1 (GFAT-1), the flux-generating step of HBP (Fig. 4), thus allowing for the down-regulation of such a shunt from glycolysis under low glucose situations [63], while chronic hexosamine flux stimulates fatty acid oxidation by activating AMPK [64]. However, regulatory pathways under AMPK control are not solely intended to divert metabolic fluxes. Rather, AMPK regulation of GSK-3β allows the concomitant regulation of inflammatory cytokine production, since the inhibition of GSK-3β elicits the deinhibition of HSF-1, thus triggering the expression of HSP70, which is an intracellular anti-inflammatory protein.

It is of note that, besides the now classical molecular chaperone action, the most remarkable intracellular effect of HSP70 is the inhibition of NF- κB activation, which has profound implications for immunity, inflammation, cell survival and apoptosis. HSP70 blocks nuclear factor κB (NF—κB) activation at different levels. For instance, HSP70 inhibits the phosphorylation of inhibitor of κB (IκBs), while heat-induced HSP70 protein molecules are able to directly bind to IκB kinase gamma (IKKγ) thus inhibiting tumor necrosis factor- α (TNFα)-induced apoptosis [65]. In fact, the supposition that HSP70 might act intracellularly as a suppressor of NF- κB pathways has been raised after a number of seminal discoveries in which HSP70 was intentionally induced, such as the inhibition of TNFα-induced activation of phospholipase A_2 in murine fibrosarcoma cells [66], the suppression of astroglial iNOS expression paralleled by decreased NF— κB activation [67] and the protection of rat hepatocytes from TNFα-induced apoptosis by treating cells with the nitric oxide (NO)-donor SNAP, which reacts with intra-

http://dx.doi.org/10.5772/55349

cellular glutathione molecules generating S-nitrosoglutathione (SNOG) that induces HSP70, and, consequently, HSP70 expression [68].

HSP70 confers protection against sepsis-related circulatory fatality via inhibition of iNOS (NOS-2) gene expression in the rostral ventrolateral medulla through the prevention of NF-κB activation, inhibition of IκB kinase activation and consequent inhibition of IκB degradation [69]. This is corroborated by the finding that HSP70 assembles with liver NF-κB/IκB complex in the cytosol thus impeding further transcription of NF-κB-dependent TNF-α and NOS-2 genes that worsen sepsis [70]. This may also be unequivocally demonstrated by treating cells or tissues with HSP70 antisense oligonucleotides that completely reverse the beneficial NF-κB-inhibiting effect of HSP70 and inducible HSP70 expression (see [68,69]). Hence, HSP70 is anti-inflammatory per se, when intracellularly located, which also explains why cyclopentenone prostaglandins (cp-PGs), which are the most powerful physiological inducers of HSP70 by activating HSF-1, are at the same time powerful anti-inflammatory autacoids [71-73].

Another striking effect of HSP70 is the inhibition of apoptosis. The intrinsic apoptotic pathway is characterized by the release of mitochondrial pro-apoptotic factors and activation of caspase enzymes, while stimulation of cell surface receptors triggers the extrinsic death-pathway. The inhibitory potential of HSP70 over apoptosis occurs via many intracellular downstream pathways (e.g. JNK, NF-κB and Akt), which are both directly and indirectly blocked by HSP70, or through inhibition of mitochondrial Bcl-2 release. Together, these mechanisms are responsible for HSP70's anti-apoptotic function in stressed-cells [74].

In conclusion, intracellularly activated HSPs of the 70-kDa family are cytoprotective and anti-inflammatory by avoiding protein denaturation and excessive NF-κB activation which may be damaging to the cells [75]. These observations link energy sensing (AMPK) to anti-inflammation (HSP70) and points out to the complexity of the impact of metabolic regulation for cell survival and function. In addition, expression of cytokines such as interleukin-1β (IL-1β), tumour necrosis factorα (TNFα), and interferon-γ (INF-γ) in pancreatic islets is important in inflammation and progression of both T1 and T2DM, and is associated with β-cell dysfunction and death. Therefore, agents or nutrients that promote anti-inflammatory responses may be beneficial as anti-diabetic therapies. Since interaction of the immune system with pancreatic islets is central to T1DM and is becoming increasing linked to T2DM, the precise mechanisms of pancreatic cell death in relation to immunological function will now be discussed.

4. Immune-like characteristics of β-cells and response to cytokines

The pathophysiology of pancreatic islets in T1 and T2DM is characterised by an inflammatory process that includes immune cell infiltration, presence of apoptotic cells, expression of cytokines or adipokines and even amyloid deposits [76]. Although the aetiology of T1DM differs from T2DM, a common feature of both is an immune system-mediated destruction of pancreatic β-cells, ultimately leading to pancreatic dysfunction and reduced β-cell mass. However, the immunological-mediated attack does not solely originate from invading

macrophages and/or cytokines produced by T-lymphocytes, as initially occurs in early stage T1DM. In fact, it also stems from local production of pro-inflammatory cytokines by the pancreatic β-cells themselves. The similarity between pancreatic β-cells and immune cells is an intriguing characteristic. Both can release and respond to cytokines; their function is dependent on changes in concentration of ROS/RNS and they both express high levels of pro-inflammatory proteins such as NFκB, iNOS, NOX and TLR's. Pancreatic β-cells have been shown to express biologically active cytokines like the pro-inflammatory cytokine IL-1β in hyperglycaemic conditions [77,78]. Due to their potent effects, cytokine production is stringently regulated. Control mechanisms include down-stream activation/processing (conversion of pro-IL-1β to IL-1β by inflammasomes), and co-expression of binding proteins/antagonists (like the IL-1 receptor antagonist, IL-1Ra), that regulate cytokine bio-reactivity [76]. However, expression of the biologically active form of IL-1β was evident in pancreatic β-cells, indicating that similar to immune cells, these cells possess the necessary cellular machinery to allow expression of immunologically active cytokines [77]. Autocrine production of IL-1β, has been correlated with autoimmune destruction of β-cells in T1DM and is also associated with glucotoxicity in the pathogenesis of T2DM patients [76,79]. IL-1β elicits its potent cytotoxic effects through activation of NFκB, and a subsequent initiation of the extrinsic cell-death pathway [78]. Additionally, chronic exposure to IL-1β results in increased iNOS expression, and consequently excess NO production. High levels of NO inhibit mitochondrial ATP synthesis and up-regulate the expression of pro-inflammatory genes in β-cells, which may potentiate β-cell failure [78].

Similar to macrophages and dendritic cells, β-cells also express TLR's that normally function to regulate the immune system [80]. TLR's interact with a wide variety of pathogen-related molecules, including lipopolysaccharide (LPS), a component of bacterial cell walls. This allows phagocytosis of microbes before infection can be established. However, in β-cells, it is believed that TLR's play a role in insulin-resistance and inflammation in T2DM. TLR2 and TLR4 have been suggested as receptors for fatty acids, and may alter insulin signalling during dyslipidaemia. We have shown that β-cells express a range of TLR's and could indeed respond to LPS via TLR's, and this interaction decreased insulin exocytosis accordingly [80]. However, glutamine restored insulin release. Glutamine can also regulate pro-inflammatory gene expression in mononuclear cells [11,80]. Glutamine also up-regulates nuclear factor of activated T cells (NFAT), and thus promotes β-cell growth, while suppressing β-cell death. Mutations in NFAT-dependent genes have been demonstrated to result in hereditary forms of T2DM [11]. Moreover, as discussed above, glutamine can enter HBP thus regulating GSK-3β activity and HSP70 expression which promotes anti-inflammation and cytoprotection [53,54].

Pancreatic β-cells are also reported to express other cytokines, including IL-6, IL-8, granulocyte colony-stimulating factor (G-CSP) and MIP-1 (macrophage inflammatory protein-1) that not only induce apoptotic β-cell death, but also signal patrolling macrophages, enhancing islet immune cell infiltration [76]. Macrophages, monocytes, neutrophils and dendritic cells perform their function by engulfing invading foreign matter including bacteria or dead cells, and degrade them using super oxide (O_2^-) generated from plasma membrane-bound NOX [27].

http://dx.doi.org/10.5772/55349

β-Cells also express NOX in large quantities, and utilise controlled NOX-derived ROS to drive mitogenic signalling and proliferation [27]. However, during hyperglycaemia or dyslipidaemia as occurs in T2DM, levels of NOX-derived ROS may increase and overwhelm antioxidant defences, leading to mitochondrial dysfunction, DNA oxidation, lipid peroxidation and β-cell death.

These reports illustrate the immune-like characteristics of pancreatic β-cells and clearly demonstrate the ability of these cells to not only respond to cytokines, but to be capable of producing endogenous cytokines in an autocrine fashion. This suggests that the immune system plays an integral part in progression of DM and may offer potential therapeutic targets. However, to develop immune-related treatments, more research is required into understanding the mechanisms of islet inflammation in both T1 and T2DM.

5. Islet inflammation in T1DM and T2DM

T1DM is exclusively an autoimmune form of DM, and islet inflammation is characterised by the presence of leukocyte infiltrates that include B-cells, T-cells, macrophages and Natural Killer (NK) cells [81]. Macrophages play a critical role since they phagocytose apoptotic and necrotic β-cells, as well as produce ROS and cytokines (TNFα, INF-γ and IL-1β), that can promote β-cell death, which leads to patient insulin-dependence. However, effector CD4-helper and CD8-cytotoxic T-cells represent the predominant pancreatic infiltrate for this disease, and recent evidence has suggested that T1DM progression may be dependent on a precarious equilibrium between migration and activation of effector and regulatory T-cells (Treg) [82]. An important element in T1DM disease development is the generation of autoreactive effector T-cells that kill pancreatic β-cells through expression of Fas, lytic granules and cytokines such as INF-γ [82]. Research into formation of these autoimmune cell types is still at an early stage, and it was only definitively shown in 2012, that autoreactive effector cytotoxic-CD8 T-cells were indeed present in T1DM human pancreatic islets [81]. Furthermore, the means by which these "homicidal" immune cells are generated and go on to attack β-cells is still not fully understood. However, part of the process is believed to involve dendritic cell migration to draining lymph nodes following antigen presentation, and stimulation of autoreactive T-cell differentiation [82,83]. T-cells sub-sets such as T_h1, T_h2 and T_h17 are thus formed and they express the necessary weaponry that is responsible for β-cell death in T1DM [82], this being exacerbated by strong psychological stress [84], one of the possible triggering factors for the onset of T1DM (for review, please see [85]). Additionally, T-cell–mediated release of INF-γ and TNFα can up-regulate expression of pro-apoptotic proteins (Bim and PUMA) leading to β-cell death, along with promoting recruitment and clearance of damaged-cells by macrophages [77,86]. On the other hand, in normal individuals, activity of these autoimmune cells is normally controlled by Treg cells and it is the failure to control the action of effector T-cells that result in autoimmune disease. The mechanisms by which Treg cells prevent autoimmune attack is also not fully elucidated, but they are thought to prevent cytotoxic action of T-cells by use of contact inhibition and release of soluble signalling factors, such as IL-10 and TGFβ (transforming growth factorβ) [82]. It is also unclear whether the

precise causes of inflammation in T1DM are a consequence of T-cell failure to respond to Treg, or whether defective or low Treg numbers are to blame for disease progression. Nonetheless, the interplay between these cell populations offers a potential therapeutic strategy for T1DM treatment [82].

Interestingly, an autoimmune element has also been reported in patients with T2DM, along with the accepted thesis of insulin-resistance [76,87]. Hyperglycaemia, dyslipidaemia and low-grade inflammation (consisting of circulating inflammatory cytokines or adipokines released by adipocyte expansion), are considered important factors in the progression of T2DM and are generally present in obese individuals who are at risk of T2DM development [77]. These conditions lead to β-cell stress through a variety of processes that mainly include uncontrolled generation of ROS/RNS and cytokine-dependent initiation of death signals. Both processes combine to reduce β-cell function and decrease β-cell mass by inducing apoptotic cell death, leading to further hyperglycaemic and dyslipidaemic complications, and causing amplification of ROS/RNS generation, cytokine release and cytokine-mediated recruitment of the immune system (i.e. inflammation). These inflammatory factors are all detrimental for β-cell survival. As mentioned previously, IL-1β is elevated in the hyperglycaemic state, is increased in T1DM and is also expressed by β-cells in T2DM [77-79,88]. Moreover, concomitant down-regulation of the receptor antagonist IL-1Ra was also observed in β-cells cultured in hyperglycaemic conditions [76]. β-Cells are similar to immune cells and dysregulated expression of IL-1β in islets can cause auto-stimulation and subsequent release of IL-1β by other β-cells, via NFκB activation [76,88]. In addition, IL-1β can promote the local expression of other cytokines, for example IL-6 and IL-8. These cytokines aid in the recruitment of patrolling macrophages, which may subsequently become activated by high microenvironment levels of IL-1β and amplify IL-1β content in their own right [76]. In terms of islet inflammation, IL-1β expression and its effects on β-cell death appears to be a uniting factor, in both T1 and T2DM and is being considered a possible therapeutic target [77,89].

While inflammation is essential to maintain tissue homeostasis, it is also beneficial and allows repair of damaged organs. However, it is the presence of chronic, out of control and unchecked inflammatory factors that contribute to β-cell death and ensuing DM. Ultimately, increased local microenvironment cytokine production in islets is detrimental and understanding the mechanisms of cytokine release and regulation, and also suppression of β-cell function, will allow the development of new treatment regimens.

6. Inflammatory mediators and suppression of β-cell function

Since inflammation and β-cell death is common to both T1 and T2DM, it is reasonable to assume that shared inflammatory mediators may exist between the two conditions. It is these mediators that promote infiltration of immune cells, suppression of β-cell function, culminating in reduced insulin exocytosis and increased β-cell apoptosis. These mediators can be loosely classified into four categories, cytokines, chemokines, ROS/RNS and other inflammatory products. However, it must be noted that the activity of these modulators can be heavily

http://dx.doi.org/10.5772/55349

influenced by nutrient availability, such as in hyperglycaemia and dyslipidaemia conditions. Further to this, there is significant crossover between the molecules in these categories, and several can significantly impact on the others, indicating a complex role in both T1 and T2DM.

T1DM is an autoimmune disease and it comes as no surprise that cytokine expression is elevated in these patients [79]. Interestingly, it is becoming more evident that cytokines also play a critical role in T2DM progression, and increased levels have been reported in T2DM patients [76,87]. The most obvious source of cytokine production is from islet invading immune cells, although other researchers have illustrated that islet β-cells could also express cytokines [76,79]. Cytokine and adipokine release also occurs from adipose tissue since it expands rapidly in obese patients. Here, hypoxia also plays a key part in cytokine release due to an inflammatory response to lack of vasculature in rapidly growing adipose tissue [90,91]. Recent evidence has suggested that adipocyte invading macrophages are a significant supplier of TNFα to the circulation in obese T2DM patients, and this could be a contributing-factor that modulates inflammation in disease progression [92,93]. It is likely that all sources contribute in some way or another to elevate cytokine levels, and consequently compound inflammation in DM patients.

The main cytokines that are responsible for inflammation in T1 and T2DM, include IL-1β, TNFα, INF-γ, IL-6 and IL-8. Central to the inflammatory role of each is stimulation of stress-induced kinases, IKKβ (inhibitor of nuclear factor kappaB kinase subunit β) and/or c-JNK (c-Jun-N-terminal kinase) [90]. Activation of IKKβ leads to translocation and activation of the NFκB. This factor targets transcription of genes associated with inflammation, and can cause subsequent up-regulation and release of IL-1β, TNFα, IL-6 and IL-8 [94,95]. Therefore, the aforementioned cytokines can initiate an auto-stimulatory or feed-forward inflammatory effect through NFκB-signalling in β-cells, resulting in amplification of inflammation. IL-1β and TNFα initiate NFκB-signalling directly via association with their relative receptors (IL-1R and TNFR) [96] and can also activate the apoptotic JNK pathway indirectly by intracellular interaction of TNF receptor associated factor (TRAF) with the cytoplasmic portion of IL-1R or TNFR [97]. NFκB can play either a pro-survival or pro-death role given the correct circumstances [98]. Both NFκB and JNK are intrinsically connected, and NFκB can prevent JNK-mediated cell death, the regulatory interactions of which have been reviewed expertly elsewhere [99]. An important component of NFκB activation and function is the presence/absence of ROS/RNS. Therefore, ROS/RNS can influence NFκB-dependent cytokine expression and consequently immune response [98].

ROS/RNS can activate nuclear translocation of NFκB which promotes gene expression. However, ROS/RNS can also have an inhibitory effect when NFκB has already translocated to the nucleus [98]. The process by which ROS/RNS affects NFκB function is not entirely known but is believed to involve alteration of the NFκB catalytic site through interaction with cysteine residues, or by inhibiting specific kinase enzymes such as IκBα, that results in phosphorylation of NFκB [98]. Cellular ROS can be generated from Electron Transport Chain (ETC) respiratory complexes or from specific enzymes (e.g. NOX-mediated production for phagocytosis) [27,98]. Most notably, in hyperglycaemic/glucotoxic conditions (in T1 or T2DM), mitochondria are the major source of ROS/RNS primarily because of high oxidative phosphorylation and ATP

production [100]. As a result of unavoidable oxidative chemistry and prolonged ETC activity, superoxide (O_2^-) anions can be formed and may "leak" from the mitochondria and elicit cellular damage [100]. Additionally, excess glucose can cause increased intracellular calcium, which may enhance mitochondrial O_2^- output, but also activate NOX-derived ROS via protein kinase C (PKC) [100]. High glucose can also induce NOX activity through NADPH production from the conversion of glucose-6-phosphate to pentose leading to increased O_2^- [100]. Superoxide is a precursor reactive species and can be converted to other forms of strong oxidants including H_2O_2, and free radicals such as hydroxyl radicals and also peroxynitrite following reaction with NO [27,100]. These reactive species can cause DNA, lipid and protein damage and can also activate/regulate NFκB, who in turn can promote cytokine release and increased NO/O_2^- production by activation of iNOS and NOX expression [100,101]. Thus, ROS/RNS can exacerbate the immunological response and lead to cell death in a cyclic fashion (Fig. 5).

Lipid- and adipose-derived factors are considered other inflammatory mediators. In T2DM patients, dyslipidaemia occurs along with hyperglycaemia and consequently vascular circulation and intracellular accumulation of lipids can have a profound effect on the inflammatory response. Excess fatty acids can induced ROS generation through increased TCA metabolite production, increased NADH/NAD+ ratio and elevated intracellular Ca^{2+} [100]. They can also increase O_2^- and NO production via activation of NOX and iNOS, respectively, all potentially activating the NFκB pathway [97,100,102]. Formation of ceramide from long chain fatty acids also contributes to precipitation of lipotoxicity in β-cells and results in ROS generation and apoptotic death [97,100]. Ceramide, synthesised by serine palmitoyltransferase from long chain fatty acids like palmitic acid [100], is capable of inhibiting the pro-survival PI3K pathway, activating caspase-9 [100]. Like other fatty acids, ceramide can associate with and activate TLR's, which may elicit an immune response [90].

Since adipose tissue expands in obese patients, increased adipose-derived factors have been detected in patient serum, including leptin, TNFα and IL-6. Leptin and adiponectin can play a role in the immune reaction in DM patients. Leptin, an appetite control endocrine factor, inhibits feeding by interaction with receptors in the hypothalamus and a subsequent stimulation of neurotransmitter release, for example norepinephrine [103]. It is considered a cytokine due to its homology in structure with IL-6, and its receptor-mediated effects [77,103,104]. It has been shown to induce β-cell death by up-regulating IL-1β, and has also been implicated in exacerbation of T1DM in animal models [77,105]. Conversely, adiponectin is considered an anti-inflammatory protein, and enhances IL-1Ra and IL-10 expression [90,106], leading to reduced IL-1β and enhanced suppression of T-cell mediated inflammation.

Chemokines can also be secreted from adipose tissue and are elevated in the adipose tissue of obese mice and humans [90,107]. CC-chemokine ligand-2 (CCL-2) functions to recruit monocytes to adipose tissue resulting in differentiation into activated macrophages [108]. Others such as CCL-3, CCL-6, CCL-7, CCL-8 and CCL-9, have also been reported to be elevated in high-fat fed mice, suggesting they may play a role in immune cell recruitment and inflammation [90].

Several mechanisms and modulators may contribute to the inflammatory response observed in T1 and T2DM. Cytokines, ROS and NFκB-signalling appear to be critical in mediating immune cell infiltration and further cytokine production. The balance between β-cell survival

http://dx.doi.org/10.5772/55349

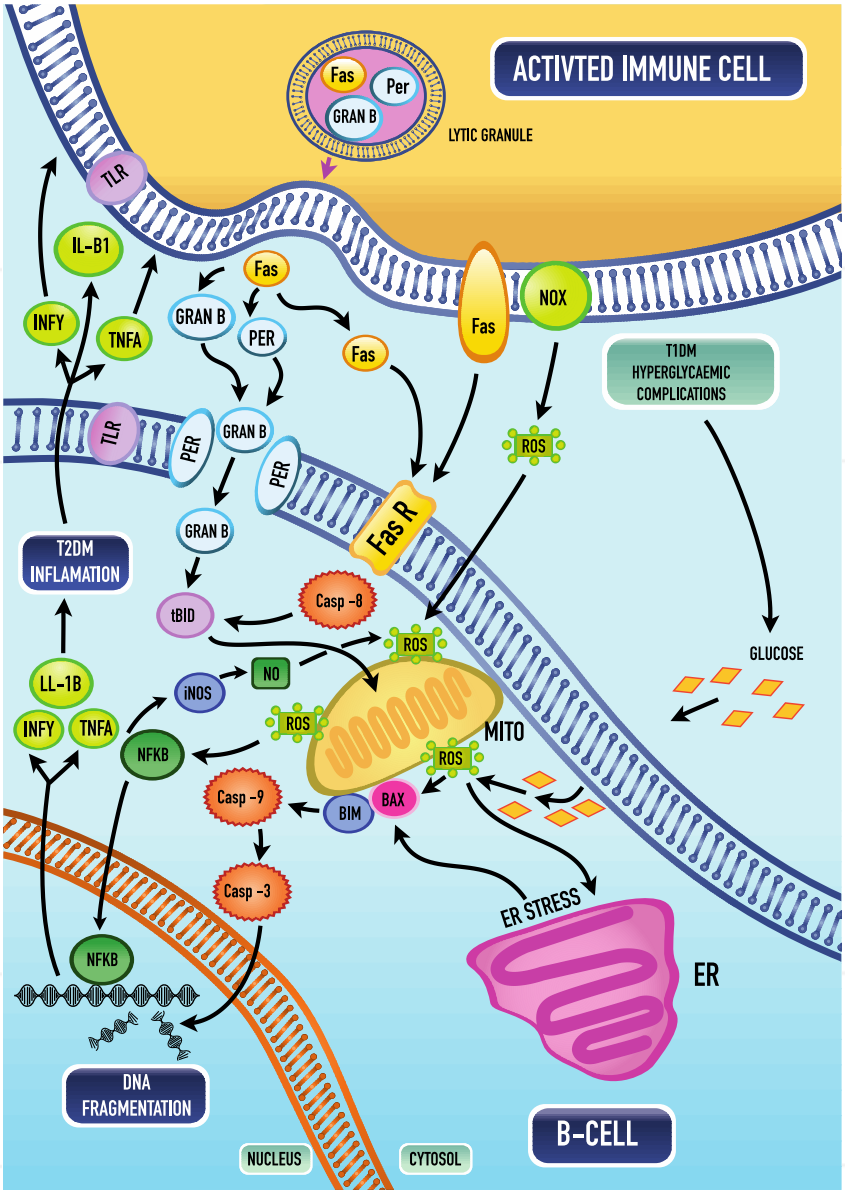

Figure 5. Illustration depicting the potential convergence points of the immunological NFκB and the metabolic ROS/ER stress pathways in pancreatic β-cells. Islet inflammation is characterised by the presence of leukocyte infiltrates that mediate the destruction of β-cells by release of cytokines, generation of ROS (NO, cytokine-NFκB-dependent]) and by activating the granzyme b- and death-receptor-mediated death pathways. Also shown is the effect of excess glucose on ROS production and ER stress that ultimately activates caspase enzymes via mitochondrial- and ER-mediated death pathways. ROS/RNS can also activate and regulate the NFκB stress pathway, which may lead to expression of cytokines that promotes immune cell infiltration exacerbating β-cell death.

and death is dependent on the interactions of these mediators, but also on the glycaemic and lipidaemic environment. We will now discuss the precise mechanisms of β-cell death in T1 and T2DM, and examine the commonalities between both.

7. Pancreatic β-cell failure and death in T1DM

Pancreatic β-cell failure can be defined as a reduction in insulin secretion or a failure to respond to plasma glucose (i.e. insulin-resistance). β-cell dysfunction in T1DM is characterised by an autoimmune-mediated destruction of β-cells leading to a decrease in pancreatic β-cell mass and reduced insulin secretion [109]. On the other hand, progression of T2DM is more variable and β-cell death occurs against the backdrop of insulin-resistance [109]. Here, the pathogenesis of T2DM usually involves a response to increased metabolic load by increased β-cell mass and enhanced insulin secretion [110]. A period of normoglycaemia ensues before a reduction in insulin secretion and β-cell function is observed. Finally, this phase is followed by a decrease in β-cell mass due to apoptotic cell death and is referred to as overt diabetes [110,111]. The shared feature associated with both T1 and T2DM is the failure of pancreatic β-cells resulting in reduced cell mass, dysfunction and ultimately apoptotic death. While there are commonalities associated with both types, the main mechanism of cell death associated with T1DM is immune-related.

At the time of diagnosis, T1DM patients present with a 70-80% reduction β-cell mass [109]. Insulitis and infiltration of leukocytes into islets is common in these patients. Several types of leukocytes are present including B-cells, macrophages and Natural Killer (NK) cells, but the principal invading cell type is the cytotoxic T-cell (CD4 and CD8) [81,82]. Immune cells promote β-cell death via several mechanisms, and these can be simplified to include phagocytosis, production of cytokines and T-cell-induced initiation of death-receptor-mediated apoptosis. The intracellular generation of ROS/RNS and activation of caspase enzymes occurs inside target cells and ultimately seals the fate of these cells.

Generation of autoreactive effector T-cells is extremely important in the pathogenesis of T1DM, but the precise biochemical mechanisms behind release of self-antigen and development of autoreactive T-cells remains unknown. However, work in this field has identified a potential role for decreased expression of peripheral tissue antigens (PTA) in pancreatic draining lymph nodes, which possibly allows unchallenged escape of differentiated autoreactive T-cells [82,83,112]. These T-cells kill pancreatic β-cells through expression of Fas ligand and expression of extracellular cytotoxic factors including cytokines, and lytic granules containing granzyme B and perforin [113]. In death-receptor-mediated apoptosis, Fas ligand or TNFα initiates death signals through association with FasR (Fas receptor) or TNFR (tumour necrosis factor receptor). An intracellular conformational change occurs that results in activation of caspase-8 [114,115]. This in turn serves to activate caspase-3 downstream promoting the apoptotic cascade [116].

T-cell-mediated release of granzyme B and perforin also leads to caspase activation in target cells. Here, perforin creates pores in the plasma membrane of the target cell, while granzyme

http://dx.doi.org/10.5772/55349

B is released into the cytosol and activates caspase-3 [113,117]. Interestingly, in order to yield activation of caspase-3, both caspase-8 from the death-receptor pathway outlined above, and granzyme B converge and initiate the mitochondrial-mediated death pathway via cleavage of BID [a member of the B-cell lymphoma-2 (Bcl-2) family of proteins], to truncated BID (tBID). In this process, cytosolic tBID translocates to the mitochondrial membrane and activates other Bcl-2-related proteins, such as BAX. Release of cytochrome *c* is then stimulated, which acts as the trigger for mitochondrial-mediated activation of caspase -9 and -3 [118,119,120]. Therefore, both the death-receptor and granzyme B-mediated death pathways activate the mitochondrial-mediated death pathway.

Conversely, macrophages induce β-cell death through production of ROS, cytokines and eventually phagocytosis. Macrophages express high levels of NOX, and use O_2^- to kill invading organisms or possibly damaged β-cells. Expression of high amounts of ROS/RNS or reduced antioxidant defences, results in mitochondrial dysfunction, which can culminate in mitochondrial-mediated apoptosis. Briefly, this involves major structural changes caused by ROS/RNS-mediated lipid/protein oxidation on both the inner and outer mitochondrial membranes, thus increasing the membrane permeability to proteins [121]. This is regulated by the interaction of pro- and anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-X_L, BAX, BAK, BIM, BID and BAD) [122]. The release of cytochrome *c* to the cytosol and its association with apoptosis protease activation factor-1 (Apaf-1) and pro-caspase-9, forms a heptameric wheel-like caspase-activating complex, known as the apoptosome, which subsequently leads to activation of caspase-9 and effector caspase-3, further down-stream [123]. Caspase activation promotes cell death by degradation of DNA and cytoskeletal proteins [124].

In addition, immune cells release cytokines (e.g. TNFα, INF-γ and IL-1β) that also promote up-regulation of ROS/RNS via activation of NFκB (e.g. NO), who in turn can be regulated by ROS [100,101]. Induction of NO expression can cause activation of tumour suppressor protein (p53) leading to inhibition of cell cycle and death [109]. Cytokines can also inflict cell death via stimulation of the JNK pathway [97]. Here, IL-1β and TNFα activate mitochondrial translocation of JNK, who is a regulator of Bcl-2 proteins. JNK phosphorylates BIM, which results in the release of BAX-dependent cytochrome *c* and initiation of mitochondrial-mediated apoptosis [125,126]. Additionally, release of INF-γ by T-cells, can also phosphorylate BIM in β-cells, promoting cell death in a similar manner [77,86].

A variety of biochemical signalling pathways are available by which autoimmune cells utilise to initiate β-cell destruction. Consequently, due to a complete lack of insulin secretion and subsequent diminished glucose-uptake by muscle and adipose tissue, hyperglycaemia ensues in T1DM patients. High levels of blood glucose leads to further complications including, glucotoxicity, lipotoxicity and glucolipotoxicity and these are key players in exacerbation of the disease, and can lead to the clinical complications of T2DM [108]. Therefore, the precise way in which these factors affect β-cell turnover and survival will be discussed in the next section. Nonetheless, β-cell death in T1DM is based on classical immune-related death processes, but also relies on involvement of ROS and mitochondrial mediated which may occur in a sub-population of beta cells in T2DM.

8. Pancreatic β-cell failure and death in T2DM

Failure of pancreatic β-cells is essential in the progression of both T1 and T2DM. The development of T2DM is more gradual than T1, and appears to occur in specific stages. It is dependent on the establishment of insulin-resistance and displays increased degrees of variability in comparison with T1DM. Therefore, determination of the precise mechanisms of T2DM-related cell death remains difficult and, these are still not fully understood.

T2DM patients have a 30-50% reduction in β-cell mass on average post-mortem and the primary candidate pathways leading to β-cell apoptosis are oxidative stress, ER stress, amyloid accumulation, ectopic lipid deposition, lipotoxicity and glucotoxicity [127]. These stresses can all be caused by over-nutrition and neatly connects T2DM to obesity [90]. Glucose, the most important insulin secretatogue, is also the most important regulator of β-cell mass and function [128]. Impaired glucose-tolerance and hyperglycaemia are hallmarks of T2DM and prolonged glucose exposure can promote glucose-desensitisation, decreased insulin secretion and generation of oxidative stress in β-cells [128]. Consequently, glucotoxicity plays a significant part in pancreatic β-cell death in T2DM.

Understandably, excess glucose increases β-cell glucose metabolism and oxidative phosphorylation. Elevated ETC activity promotes increased superoxide (O_2^-) anion leakage from the mitochondria and may cause oxidative cellular damage [100]. Furthermore, high glucose levels induces NOX activity via NADPH production from metabolism of glucose to pentose, and through the TCA cycle, both leading to increased O_2^- output [100]. O_2^- can be converted to the less reactive H_2O_2 via superoxide dismutase, to the highly reactive hydroxyl anion by the iron-catalysed Fenton reaction, or to peroxynitrite ($ONOO^-$) via reaction with iNOS-derived NO [27,129]. β-cells are considered vulnerable to ROS/RNS generation because they express relatively low levels of antioxidant enzymes, like glutathione and catalase [27,128,129]. These enzymes immediately convert H_2O_2 to molecular oxygen and water. However, the detrimental combination of reduced activity of antioxidant enzymes, along with ROS/RNS generation can result in oxidative damage to DNA, lipids and proteins, thereby promoting mitochondrial-mediated apoptosis. In addition, ROS/RNS can also activate and regulate biochemical stress pathways, such as the NFκB, leading to further negative effects in β-cells [100,101].

Excess glucose can cause increased intracellular calcium, as outlined previously, which may enhance mitochondrial O_2^- production, but also deplete ER Ca^{2+}, promoting activation of the ER-stress-mediated death pathway [111,128] alongside unfolded protein response (UPR) (for review, please see [130]). In normal conditions, proteins are synthesised in the ER and are subsequently secreted or routed into a variety of sub-cellular compartments. However, accumulation of native or unfolded proteins within the lumen of the ER can activate caspase enzymes and ultimately promote cell death [131,132]. Reaction of ROS with the ER leads to protein accumulation via dysregulation of the ER oxidative folding pathway [111]. It also results in oxidative activation of Ca^{2+} release channels in the ER membrane, thereby depleting the ER of Ca^{2+} [111]. This ER stress activates pro-caspase-12, located on the cytoplasmic side of the ER, in a manner similar to caspase-9 [133,134]. Caspase-12 apoptosomes also causes translocation of JNK to the mitochondrial membrane inducing BIM

http://dx.doi.org/10.5772/55349

phosphorylation, ultimately leading to cytochrome *c* release and initiation of mitochondrial-dependent apoptosis [111,135,136].

Lipid accumulation (lipotoxicity) in the ER may also play a significant function in mediating ER stress in β-cells. Obesity is a primary risk factor associated with T2DM, and is accompanied with increased plasma glucose and lipid levels due to high carbohydrate- and fat-based diets [137]. The process by which free fatty acids modulate ER stress is not entirely known [111] but, it has been shown that palmitic acid could deplete ER Ca^{2+} levels and augment ER morphology and integrity, which may cause activation of ER stress by the mechanisms mentioned above [137,138]. Furthermore, excess fatty acid esterification in the ER, may divert ER machinery and delay the processing and export of proteins in the ER [137]. Since there is a large demand for protein/insulin production in pancreatic islets, β-cells have a highly active and well developed ER. This suggests that β-cells may be more susceptible to ER stress during protein synthesis [109,137]. Given the effects of elevated plasma glucose and lipids in T2DM patients, ER stress could be a vital mechanism facilitating glucotoxicity-, lipotoxicity- or glucolipotoxicity-mediated β-cell failure and death [137].

Moreover, accumulation of islet amyloid polypeptide (IAPP) may also contribute to β-cell dysfunction and death in a manner similar to that described above [110,111,139]. IAPP precipitates into lethal oligomers inside the ER and like unfolded proteins, activates the ER stress-mediated death pathway [139]. IAPP deposits are present in over 90% of T2DM islets, post-mortem, indicating a substantial participation in T2DM progression [109,111].

In summary, several biochemical mechanisms have been suggested to be responsible for pancreatic β-cell failure and death in T2DM. However, there appears to be significant interplay between the purported pathways and conditions of glucotoxicity-, lipotoxicity- and glucolipotoxicity, which are common in the aetiology of T2DM and require further investigation. Interestingly, from this review there are noteworthy commonalities associated with T1 and T2DM mechanisms of β-cell failure and death. In the following section we will attempt to summarise these, with a view to identifying the potential therapeutic targets that are of interest to the research community.

9. Similarities between β-cell failure and death in T1DM and T2DM

T1DM is considered a chronic autoimmune disease and the major mechanisms responsible β-cell death and dysfunction are immune-related. In contrast, the main mechanisms responsible β-cell death and dysfunction in T2DM are related to metabolism. Thus, the convergence points of these two aetiologically-different disorders appear to be the immunological NFκB pathway and, the metabolic ROS/ER stress pathways (Fig. 5).

Islet inflammation in T1DM is characterised by the presence of leukocyte infiltrates [81]. In particular, macrophages and T-cells mediate the destruction of β-cells by phagocytosis, release of cytokines, generating ROS (NO, cytokine-NFκB-dependent [82]) and by activating the granzyme b- and death-receptor-mediated death pathways (Fig. 5). At the biochemical level,

production of cytokines such as INF-γ, TNFα and IL-1β act in synergy to promote expression of iNOS and consequently NO, via stimulation of NFκB in mouse islet β-cells [82]. If not regulated, this generation of ROS may impact on ER stress and possibly promote cell death, which has been shown to be an import cell death process in T2DM (Fig. 5). Furthermore, cytokine–mediated activation of β-cell NFκB may result in an autocrine production of similar cytokines by β-cells, amplifying these death signals [76,79]. Another complication that arises with T1DM and immune-mediated reduction of β-cell mass is impaired insulin secretion, which may possibly promote additional hyperglycaemia and dyslipidaemia in these patients. Therefore, and as explained earlier, glucolipotoxicity may follow, along with further cell death that is achieved through mitochondrial- and/or ER-mediated death processes (Fig. 5).

In T2DM, hyperglycaemia and dyslipidaemia are critical factors and are generally present in obese individuals with the disease [77]. Consequently, excess glucose and circulating free fatty acids may promote increased ROS production and ER stress by enhancing oxidative phosphorylation and causing a build-up of unfolded proteins in the ER. Elevated ROS and ER stress will activate caspase enzymes via mitochondrial- and ER-mediated death pathways, respectively (Fig. 5). Interestingly, ROS/RNS can also activate and regulate the NFκB stress pathway, which may possibly lead to transcription of genes coding either cytokines or immune cell chemo-attractants (Fig. 5) [98]. Given the spontaneous reactivity of ROS, it is not clear yet exactly how it influences NFκB activation. However, it has been shown to react in a variety of ways promoting stimulation or inhibition of NFκB, with effects dependent on context [98]. For example, if ROS does promote expression of NFκB-derived cytokines or immune cell chemo-attractants, these signals may alert nearby macrophages and T-cells to the elevated level of β-cell ROS, and initiate the removal of these damaged cells. Consequently, this could result in immune cell infiltration into pancreatic islets of T2DM patients and possibly β-cell death (Fig. 5).

Interestingly, an autoimmune element has been reported in obese patients with T2DM, who have presented with elevated circulatory cytokine and acute-phase protein levels [77,87]. A common denominator that may link both T1 and T2DM is IL-1β. Autocrine production of IL-1β by β-cells has been observed in T1DM and in T2DM patients [76,79]. Furthermore, *in vitro* culture of islets from non-diabetic doners in high glucose, caused increased production and secretion of IL-1β, along with subsequent NFκB activation, Fas up-regulation, reduced insulin secretion and β-cell DNA fragmentation [77,78]. Chronic exposure to IL-1β also increases expression of iNOS, and consequently may up-regulate ROS generation and the expression of other pro-inflammatory cytokines like IL-6 and IL-8, which may further potentiate β-cell failure [78]. These reports clearly demonstrate the inherent link between glucotoxicity and the inflammatory processes [77]. In addition, investigators took a step further and showed that exogenous addition of IL-1Ra, the IL-1 receptor antagonist (Anakinra), protected the islets from IL-1β, but also reduced glycated haemoglobin in a small clinical trial of T2DM patients [77,140]. Clinical trials utilising IL-1Ra, still continue [90].

In conclusion, T1 and T2DM are different diseases, but do appear to share some common biochemical ground in terms of disease development. Although T1DM is mostly an autoimmune-related syndrome, elements of metabolic dysregulation are evident. Likewise, even

http://dx.doi.org/10.5772/55349

though T2DM is very much a metabolic disease, there are also immunological-related factors that may exacerbate disease progression. NFκB, IL-1β, ER-stress and generation of ROS/RNS are instrumental players in both diseases, and may warrant further investigation with regard to development of novel therapies.

10. β-cell therapies and possible targets for prevention of β-cell failure

The traditionalistic concept of separate T1 and T2DM syndromes has become clouded with knowledge of the involvement of inflammation in T2DM and the metabolic syndrome in T1DM [108]. It is now apparent that treatment modalities that were specifically designed for one form of diabetes may have application in the other. Exercise, weight loss and diet are the most effective strategies to delay T2DM disease development, but similar strategies have shown significant efficacy in T1DM [108,128].

Researchers have targeted TNFα in children with newly diagnosed T1DM and showed that a recombinant TNFR fusion protein preserved c-peptide function, along with enhancing insulin production [82,141]. However, to date, anti-TNFα treatment has failed to improve blood glucose in T2DM patients [90]. Infiltration of cytotoxic T-cells in T1DM has been well characterised [82]. Therefore, some developing treatment strategies for this precise component of T1DM disease is the generation of T-cell targeted therapy to prevent the destruction of transplanted islets, some of which include introduction of anti-inflammatory Tregs that regulate T-cell activation [89]. Since inflammation has been detected in T2DM, these approaches may have similar applications. Directing treatment towards the immunological pathways is quite attractive and recent evidence has suggested that the most promising results involve blockade of IL-1β or NFκB activation [90]. Again, it is noteworthy to highlight that enhanced HSP70 expression has been convincingly demonstrated to protect against obesity-induced insulin-resistance [142], while low HSP70 contents in skeletal muscle of T2DM patients are associated with insulin-resistance [143,144]. Hence, pharmacological (e.g. the hydroxylamine derivative BGP-15, now under clinical trial) as well as physiological (hyperthermic, hot tube) treatments have started to be cogitated as promising therapeutic approaches in T2DM [142,145]. Moreover, physical exercise, which is a powerful antidiabetic intervention, is one of the strongest ways to increase intracellular HSP70 expression in many tissues (for reviews, please see [75,146]), including in pancreatic β-cells (A. Bittencourt et al., manuscript in preparation).

Elevated IL-1β and reduced IL-1Ra is known to correlate with T1DM, but the recent identification of inflammation in T2DM has meant that the IL-1 receptor antagonist (Anakinra), has been trialed in both T2DM and T1DM patients with successful results [77,140,147]. Here, the agent lowered blood glucose, reduced inflammation, improved insulin-sensitivity and secretion. These reports again illustrate the pivotal role played by IL-1β in mediating DM development, and thus clinical trials continue [90].

Salicylate-derivatives, such as salsalate, are also being used in an anti-inflammatory capacity to inhibit the activation of NFκB, although the precise mechanisms of action are not fully

understood. These agents have the clear advantages of being orally available and well tolerated. Salsalate has been shown to improve insulin sensitivity and production, increase secretion of the anti-inflammatory cytokine adiponectin, reduce blood glucose and C-reactive protein (CRP) and decrease fatty acid and triglyceride levels [90].

From our own studies we have shown how different amino and fatty acid combinations may affect β-cell metabolism. This proposes the concept of diet manipulation as an additional treatment for hyperglycaemia and lipidaemia in T2 and even T1DM patients. We demonstrated the antioxidant activities of arachidonic acid, arginine and glutamine, and this data may suggests that dietary supplementation, high in specific amino or fatty acids, may have favourable effects in DM patients. Given the role of ROS and ER stress in β-cell death, dietary or pharmacological agents that target these pathways may also represent novel treatments for the delay or prevention of DM.

11. Conclusions and perspectives

Over-nutrition and diminished physical activity in the modern lifestyle has led to a staggering increase in T2DM onset in Western cultures [108]. However, the epidemic is also progressing into the developing world, indicating that T2DM has become a major global health issue [108]. Since the 1990's, T1DM has more than doubled in number and is expected to double again before 2020 [108,148]. The traditional classification of distinct criteria for T1 and T2DM syndromes has become blurred due to the global increase in obese individuals and the incidence of obesity-related insulin-resistance [108]. Currently the paradigm of T1 and T2DM treatment appears to be changing in line with the clarification of dysfunctional pathways that are common to both disease types. Although diet-and-exercise still remains the most effective (and cheapest) treatment, new therapies will be required going into the future. Consequently, an increased understanding of the molecular and biochemical mechanisms that lead to disease onset and progression are mandatory.

In this manuscript, we have examined some of the key pathways that are essential in the pathogenesis of both T1 and T2DM, and we have reviewed some of the novel treatments that are currently being developed to counteract these dysfunctional processes. It is clear that inflammation, generation of ROS/RNS and ER stress leads to significant damage to pancreatic β-cells, culminating in cell dysfunction, and ultimately cell death. It is hoped that further study of the NFκB and the ER stress-mediated pathways, will reveal novel therapeutic targets that can be developed into a new generation of anti-diabetic treatments, that will improve β-cell function, survival and regeneration in T1 and T2DM.

Acknowledgements

The authors would like to thank Mr. Peter McEvoy for assistance with design and illustration of Figure 5.

http://dx.doi.org/10.5772/55349

Abbreviations

ACC - Acetyl coA carboxylase

ACL - ATP-citrate lyase

AMPK - AMP-activated kinase

Apaf-1 - Apoptosis protease activation factor-1

Bcl-2 - B-cell lymphoma-2

Cp-PGs - Cyclopentenone prostaglandins

CPT-1 - Carnitine palmitoyl transferase 1

DHAP - Dihydroxyacetone phosphate

ER - Endoplasmic reticulum

ETC - Electron transport chain

FasR - Fas receptor

GABA - γ-aminobutyric acid

G-CSP - Granulocyte colony-stimulating factor

GDH - Glutamate dehydrogenase

GFAT-1 - Glutamine:fructose-6-phosphate amidotransferase-1

GSIS - Glucose-stimulated insulin secretion

GSK3β - Glycogen synthase kinase-3β

HBP - Hexosamine biochemical pathway

HSF-1 - Heat shock factor-1

HSP70 - Heat shock protein-70

iNOS - Inducible nitric oxide synthase

JNK - c-Jun-N-terminal kinase

LDH - Lactate dehydrogenase

MCFs - Metabolic coupling factors

ME1 - Malic enzyme1

MIP-1 - Macrophage inflammatory protein-1

NFAT - Nuclear factor of activated T cells

NFκB - Nuclear factor κB

NOD - Non-obese diabetic

NOX - NADPH oxidase

PC - Pyruvate carboxylase

PDH - Pyruvate dehydrogenase

PP2A - Protein phosphatase type 2A

PTA - Peripheral tissue antigens

RNS - Reactive nitrogen species

ROS - Reactive oxygen species

SNARE -Soluble NH2-ethylmaleimide-sensitive fusion protein attachment protein receptor

SNOG - S-nitrosoglutathione

T1DM - Type 1 diabetes mellitus

T2DM - Type 2 diabetes mellitus

TCA - Tricarboxylic acid

TGFβ – Transforming growth factorβ

TLR - Toll-like receptors

TNFR - TNFα receptor

TRAF - TNF receptor associated factor

Tregs - Regulatory T-cells

VAMP - Vesicle-associated membrane protein

Author details

Philip Newsholme[1], Kevin Keane[1], Paulo I Homem de Bittencourt Jr.[2,3] and Mauricio Krause[4,5]

1 School of Biomedical Sciences, Curtin University, Western Australia

2 Department of Physiology, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

3 National Institute of Hormones and Women's Health, Brazil

4 Institute of Technology Tallaght and Institute for Sport and Health UCD Dublin, Ireland

5 School of Public Health and Physiotherapy, UCD Dublin, Ireland

http://dx.doi.org/10.5772/55349

References

[1] Chan, S. J, & Steiner, D. F. Insulin Through the Ages: Phylogeny of a growth promoting and metabolic regulatory hormone. American Zoologist (2000). , 40, 213-22.

[2] Irwin, D. M, Humer, O, & Youson, J. H. Lamprey Proglucagon and the origin of glucagon-like peptides. Molecular Biology and Evolution (1999). , 16(11), 1548-57.

[3] Newsholme, P, Abdulkader, F, Rebelato, E, Romanatto, T, Pinheiro, C. H, Vitzel, K. F, Silva, E. P, Bazotte, R. B, Procopio, J, Curi, R, Gorjao, R, & Pithon-curi, T. C. Amino acids and diabetes: implications for endocrine, metabolic and immune functions. Frontiers in Bioscience (2011). , 16, 315-39.

[4] Jitrapakdee, S, Wutthisathapornchai, A, Wallace, J. C, & MacDonald, M.J. Regulation of insulin secretion: role of mitochondrial signalling. Diabetologia (2010). , 53(6), 1019-32.

[5] Rutter, G. A, & Hill, E. V. Insulin vesicle release: walk, kiss, pause…then run. Physiology (Bethesda, Md.) (2006). , 21, 189-96.

[6] Gembal, M, Gilon, P, & Henquin, J. C. Evidence that glucose can control insulin release independently from its action on ATP-sensitive K+ channels in mouse B cells. Journal of Clinical Investigations (1992). , 89(4), 1288-95.

[7] Remedi, M. S, Rocheleau, J. V, Tong, A, Patton, B. L, Mcdaniel, M. L, Piston, D. W, Koster, J. C, & Nichols, C. G. Hyperinsulinism in mice with heterozygous loss of K(ATP) channels. Diabetologia (2006). , 49(10), 2368-78.

[8] Miki, T, Nagashima, K, Tashiro, F, Kotake, K, Yoshitomi, H, Tammamoto, A, Gonoi, T, Iwanaga, T, Miyazaki, J. I, & Seino, S. Defective insulin secretion and enhanced insulin action in KATP channel-deficient mice. Proceedings of the National Academy of Sciences (1998). , 95, 10402-06.

[9] Straub, S. G, & Sharp, G. W. G. Glucose-stimulated signalling pathways in biphasic insulin secretion. Diabetes and Metabolism Research and Reviews (2002). , 18(6), 451-63.

[10] Newsholme, P, & Krause, M. Nutritional Regulation of Insulin Secretion: Implications for Diabetes. The Clinical Biochemist Reviews (2012). , 33(2), 35-47.

[11] Newsholme, P, Bender, K, Kiely, A, & Brennan, L. Amino acid metabolism, insulin secretion and diabetes. The Biochemical Society Transactions (2007). Pt 5): 1180-6.

[12] Opara, E. C, Garfinkel, M, Hubbard, V. S, Burch, W. M, & Akwari, O. E. Effect of fatty acids on insulin release; role of chain length and degree of unsaturation. The American Journal of Physiology (1994). Pt 1): E, 635-9.

[13] Keane, D. C, Takahashi, H. K, Dhayal, S, Morgan, N. G, Curi, R, & Newsholme, P. Arachidonic acid actions on functional integrity and attenuation of the negative ef-

fects of palmitic acid in a clonal pancreatic β-cell line. Clinical Science (London) (2011). , 120(5), 195-206.

[14] Hosokawa, H, Corkey, B. E, & Leahy, J. L. Beta-cell hypersensitivity to glucose following 24-h exposure of rat islets to fatty acids. Diabetologia (1997). , 40(4), 392-7.

[15] Vassiliou, E. K, Gonzalez, A, Garcia, C, Tadros, J. H, Chakraborty, G, & Toney, J. H. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-α both *in vitro* and *in vivo* systems. Lipids in Health and Disease (2009).

[16] Keane, D, & Newsholme, P. Saturate and unsaturated (including arachidonic acid) non-esterified fatty acid modulation of insulin secretion from pancreatic β-cells. The Biochemical Society Transactions (2008). Pt 5): 955-8.

[17] Jensen, M. V, Joseph, J. W, Ronnebaum, S. M, Burgess, S. C, Sherry, A. D, & Newgard, C. B. Metabolic cycling in control of glucose-stimulated insulin secretion. The American Journal of Physiological Endocrinology and Metabolism (2008). E, 1287-97.

[18] Newsholme, P, Keane, D, Welters, H. J, & Morgan, N. G. Life and death decisions of the pancreatic beta-cell: the role of fatty acids. Clinical Science (London) (2007). , 112(1), 27-42.

[19] Mcclenaghan, N. H, Scullion, S. M, Mion, B, Hewage, C, Malthouse, J. P, Flatt, P. R, Newsholme, P, & Brennan, L. Prolonged L-alanine exposure induces changes in metabolism, Ca2+ handling and desensitisation of insulin secretion clonal pancreatic beta-cells. Clinical Science (London) (2009). , 116(4), 341-51.

[20] Bender, K, Maechler, P, Mcclenaghan, N. H, Flatt, P. R, & Newsholme, P. Overexpression of malate-aspartate NADH shuttle member Aralar1 in the clonal beta-cell line BRIN-BD11 enhances amino-acid-stimulated insulin secretion and cell metabolism. Clinical Science (London) (2009). , 117(9), 321-30.

[21] Newsholme, P, Brennan, L, & Bender, K. Amino acid metabolism, β-cell function and diabetes. Diabetes (2006). S, 39-47.

[22] Maassen, J. A, Hart, t, Janssen, L. M, Reiling, G. M, Romijn, EJ. A, & Lemkes, . Mitochondrial diabetes and its lesson for common type 2 diabetes. The Biochemical Society Transactions (2006). Pt 5): 819-23.

[23] Sener, A, Best, L. C, Yates, A. P, Kadiata, M. M, Oliveares, E, Louchami, K, Jijakli, H, Ladriere, L, & Malaisse, W. J. Stimulus-secretion coupling of arginine-induced insulin-release: comparison between the cationic amino acid and its methyl ester. Endocrine (2000). , 13(3), 329-40.

[24] Mcclenaghan, N. H, Barnett, C. R, & Flatt, P. R. Na^+ cotransport by metabolizable and nonmetabolizable amino acids stimulates a glucose-regulated insulin-secretory response. Biochemical and Biophysical Research Communications (1998). , 249(2), 299-303.

[25] Broca, C, Brennan, L, Petit, P, Newsholme, P, & Maechler, P. Mitochondria-derived glutamate at the interplay between branched-chain amino acid and glucose-induced insulin secretion. FEBS Letters (2003).

[26] Krause, M, Rodrigues-krause, J, Hagan, O, De Vito, C, Boreham, G, Susta, C, Newsholme, D, & Murphy, P. C. Differential nitric oxide levels in the blood and skeletal muscle of type 2 diabetic subjects may be a consequence of adiposity: a preliminary study. Metabolism: Clinical and Experimental (2012). , 61(11), 1528-37.

[27] Newsholme, P, Rebelato, E, Abdulkader, F, Krause, M, Carpinelli, A, & Curi, R. Reactive oxygen and nitrogen species generation, antioxidant defences and β-cell function: a critical role for amino acids. The Journal of Endocrinology (2012). , 214(1), 11-20.

[28] Pryor, W. A, & Squadrito, G. L. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. American Journal of Physiology (1995). Pt 1): L, 699-722.

[29] Beckman, J. S, Beckman, T. W, Chen, J, Marshall, P. A, & Freeman, B. A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proceedings of the National Academy of Sciences (1990). , 87(4), 1620-24.

[30] Crow, J. P, & Beckman, J. S. The role of peroxynitrite in nitric oxide-mediated toxicity. Current Topics in Microbiology and Immunology (1995). , 196(1), 57-73.

[31] Delaney, C. A, Tyrberg, B, Bouwens, L, Vaghef, H, Hellman, B, & Eizirik, D. L. Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death. FEBS Letters (1996). , 394(3), 300-6.

[32] Rabinovitch, A, & Suarez-pinzon, W. L. Role of cytokines in the pathogenesis of autoimmune diabetes mellitus. Reviews in Endocrine and Metabolic Disorders (2003). , 4(3), 291-9.

[33] Sancez-margalet, V, Valle, M, Ruz, F. J, Gascon, F, Mateo, J, & Goberna, R. Elevated plasma total homocysteine levels in hyperinsulinaemic obese subjects. The Journal of Nutritional Biochemistry (2002). , 13(2), 75-79.

[34] Patterson, S, Flatt, P. R, & Mcclenaghan, N. H. Homocysteine and other structurally-diverse amino thiols can alter pancreatic beta cell function without evoking cellular damage. (2006). Biochemica et Biophysica Acta 2006; , 1760(7), 1109-14.

[35] Medina, M, Urdiales, J. L, & Amores-sanchez, M. I. Roles of homocysteine in cell metabolism: old and new functions. The European Journal of Biochemistry (2001). , 268(14), 3871-82.

[36] Patterson, S, Flatt, P. R, & Mcclenaghan, N. H. Homocysteine-induced impairment of insulin secretion from clonal pancreatic BRIN-BD11 beta-cells is not prevented by catalase. Pancreas (2007). , 34(1), 144-51.

[37] Baylis, C. Nitric oxide deficiency in chronic kidney disease. The American Journal of Physiology: Renal Physiology (2008). F, 1-9.

[38] Ammon, H. P, Hehl, G, Enz, G, Setaidi-ranti, A, & Verspohl, J. Cysteine analogues potentiate glucose-induced insulin release in vitro. Diabetes (1986). , 35(12), 1390-6.

[39] Rasilainen, S, Nieminen, J. M, Levonen, A. L, Otonkoski, T, & Lapatto, R. Dose-dependent cysteine-mediated protection of insulin-producing cells from damage by hydrogen peroxide. Biochemical Pharmacology (2002). , 63(7), 1297-304.

[40] Kaneko, Y, Kimura, T, Taniquchi, S, Souma, M, Kojima, Y, Kimura, Y, Kimura, H, & Niki, I. Glucose-induced production of hydrogen sulphide may protect the pancreatic beta-cells from apoptotic cell death by high glucose. FEBS Letters (2009). , 538(2), 377-82.

[41] Kaneko, Y, Kimura, T, & Kimura, H. and Niki I. L-cysteine inhibits insulin release from pancreatic beta-cells: possible involvement of metabolic production of hydrogen sulphide, a novel gasotransmitter. Diabetes (2006). , 55(5), 1391-7.

[42] Sener, A, & Malaisse, W. J. L-l. e. u. c. i. n. e. and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature (1980). , 288(5787), 187-9.

[43] Brennan, L, Corless, M, Hewage, C, Malthouse, J. P, Mcclenaghan, N. H, Flatt, P. R, & Newsholme, P. C NMR analysis reveals a link between L-glutamine metabolism, D-glucose metabolism and gamma-glutamyl cycle activity in a clonal pancreatic beta-cell line. Diabetologia (2007). , 46(11), 1512-21.

[44] Corless, M, Kiely, A, Mcclenaghan, N. H, Flatt, P. R, & Newsholme, P. Glutamine regulates expression of key transcription factor, signal transduction, metabolic gene, and protein expression in a clonal pancreatic beta-cell line. The Journal of Endocrinology (2006). , 190(3), 719-27.

[45] Brennan, L, Shine, A, Hewage, C, Malthouse, J. P, Brindle, K. M, Mcclenaghan, N. H, Flatt, P. R, & Newsholme, P. A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic beta-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. Diabetes (2002). , 51(6), 1714-21.

[46] MacDonald MJ. and Fahien L.A. Glutamate is not a messenger in insulin secretion. The Journal of Biological Chemistry (2000). , 275(44), 34025-7.

[47] Gammelsaeter, R, Coppola, T, Marcaggi, P, Storm-mathisen, J, Chaudry, F. A, Attwell, D, Regazzi, R, & Gundersen, V. A role for glutamate transporters in the regulation of insulin secretion. PLoS One (2011). e22960.

[48] Huang, M, & Joseph, J. W. Metabolomic analysis of pancreatic β-cell insulin release in response to glucose. Islets (2012). , 4(3), 210-22.

[49] Hamaoka, R, Fujii, J, Miyagawa, J, Takahashi, M, Kishimoto, M, Moriwaki, M, Yamamoto, K, Kajimoto, Y, Yamasaki, Y, Hanafusa, T, Matsuzawa, Y, & Taniguchi, N.

Overexpression of the aldose reductase gene induces apoptosis in pancreatic beta-cells by causing a redox imbalance. The Journal of Biochemistry. (1999). , 126(1), 41-7.

[50] Gabbay, K. H, & Tze, W. J. Inhibition of glucose-induced release of insulin by aldose reductase inhibitors. Proceedings of the National Academy of Sciences (1972). , 69(6), 1435-9.

[51] Ladrière, L, Louchami, K, Laghmich, A, Malaisse-lagae, F, & Malaisse, W. J. Labeling of pancreatic glycogen by D-[U-14c]glucose in hyperglycemic rats. Endocrine (2001). , 14(3), 383-97.

[52] Doherty, M, & Malaisse, W. J. Glycogen accumulation in rat pancreatic islets: in vitro experiments. Endocrine (2001). , 14(3), 303-9.

[53] He, B, Meng, Y. H, & Mivechi, N. F. Glycogen synthase kinase 3beta and extracellular signal-regulated kinase inactivate heat shock transcription factor 1 by facilitating the disappearance of transcriptionally active granules after heat shock. Molecular and Cellular Biology (1998). , 18(11), 6624-33.

[54] Kazemi, Z, Chang, H, Haserodt, S, & Mcken, C. and Zachara NE. O-linked beta-N-acetylglucosamine (O-GlcNAc) regulates stress-induced heat shock protein expression in a GSK-3beta-dependent manner. The Journal of Biological Chemistry (2010). , 285(50), 39096-107.

[55] Srinivasan, V, Tatu, U, Mohan, V, & Balasubramanyam, M. Molecular convergence of hexosamine biosynthetic pathway and ER stress leading to insulin-resistance in L6 skeletal muscle cells. Molecular and Cellular Biochemistry (2009).

[56] Hasan, N. M, Longacre, M. J, Stoker, S. W, Boonsaen, T, Jitrapakdee, S, Kendrick, M. A, & Wallace, J. C. and MacDonald M.J. Impaired anaplerosis and insulin secretion in insulinoma cells caused by small interfering RNA-mediated suppression of pyruvate carboxylase. The Journal of Biological Chemistry (2008). , 283(42), 28048-59.

[57] Xu, J, Han, J, Long, Y. S, Epstein, P. N, & Liu, Y. Q. The role of pyruvate carboxylase in insulin secretion and proliferation in rat pancreatic beta-cells. Diabetologia (2008). , 51(11), 2022-30.

[58] Gaussin, V, Hue, L, Stalmans, W, & Bollen, M. Activation of hepatic acetyl-CoA carboxylase by glutamate and Mg^{2+} is mediated by protein phosphatase-2A. The Biochemical Journal (1996). Pt 1): 217-24.

[59] Xiang, X, Saha, A. K, Wen, R, Ruderman, N. B, & Luo, Z. AMP-activated protein kinase activators can inhibit the growth of prostate cancer cells by multiple mechanisms. Biochemical and Biophysical Research Communications (2004). , 321(1), 161-7.

[60] Shapiro, H, Shachar, S, Sekler, I, Hershfinkel, M, & Walker, M. D. Role of GPR40 in fatty acid action on the beta-cell line INS-1E. Biochemical and Biophysical Research Communications (2005). , 335(1), 97-104.

[61] Tomita, T, Masuzaki, H, Iwakura, H, Fujikura, J, Noquchi, M, Tanaka, T, Ebihara, K, Kawamura, J, Komoto, I, Kawaquchi, Y, Fujimoto, K, Doi, R, Shimada, Y, Hosoda, K, Imamura, M, & Nakao, K. Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic beta-cells and implications for insulin secretion. Diabetologia (2006). , 49(5), 962-8.

[62] Krause, M. S, Mcclenaghan, N. H, & Flatt, P. R. Homem de Bittencourt P.I., Murphy C. and Newsholme P. L-arginine is essential for pancreatic b-cell functional integrity, metabolism and defence from inflammatory challenge. The Journal of Endocrinology (2011). , 211(1), 87-97.

[63] Eguchi, S, Oshiro, N, Miyamoto, T, Yoshino, K, Okamoto, S, Ono, T, Kikkawa, U, & Yonezawa, K. AMP-activated protein kinase phosphorylates glutamine : fructose-6-phosphate amidotransferase 1 at Ser243 to modulate its enzymatic activity. Genes to Cells (2009). , 14(2), 179-89.

[64] Luo, B, Parker, G. J, Cooksey, R. C, Soesanto, Y, Evans, M, Jones, D, & Mcclain, D. A. Chronic hexosamine flux stimulates fatty acid oxidation by activating AMP-activated protein kinase in adipocytes. The Journal of Biological Chemistry (2007). , 282(10), 7172-80.

[65] Ran, R, Lu, A, Zhang, L, Tang, Y, Zhu, H, Xu, H, Feng, Y, Han, C, Zhou, G, Rigby, A. C, & Sharp, F. R. Hsp70 promotes TNF-mediated apoptosis by binding IKK gamma and impairing NF-kappa B survival signaling. Genes and Development (2004). , 18(12), 1466-81.

[66] Jaattela, M. Overexpression of major heat shock protein hsp70 inhibits tumor necrosis factor-induced activation of phospholipase A2. The Journal of Immunology (1993). , 151(8), 4286-94.

[67] Feinstein, D. L, Galea, E, Aquino, D. A, Li, G. C, Xu, H, & Reis, D. J. Heat shock protein 70 suppresses astroglial-inducible nitric-oxide synthase expression by decreasing NFkappaB activation. The Journal of Biological Chemistry (1996). , 271(30), 17724-32.

[68] Kim, Y. M, De Vera, M. E, Watkins, S. C, & Billiar, T. R. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-alpha-induced apoptosis by inducing heat shock protein 70 expression. The Journal of Biological Chemistry (1997). , 272(2), 1402-11.

[69] Chan, J. Y, Ou, C. C, Wang, L. L, & Chan, S. H. Heat shock protein 70 confers cardiovascular protection during endotoxemia via inhibition of nuclear factor-kappaB activation and inducible nitric oxide synthase expression in the rostral ventrolateral medulla. Circulation (2004). , 110(23), 3560-66.

[70] Chen, H. W, Kuo, H. T, Wang, S. J, Lu, T. S, & Yang, R. C. In vivo heat shock protein assembles with septic liver NF-kappaB/I-kappaB complex regulating NF-kappaB activity. Shock (2005). , 24(3), 232-8.

[71] Rossi, A, Kapahi, P, Natoli, G, Takahashi, T, Chen, Y, Karin, M, & Santoro, M. G. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature (2000). , 403(6765), 103-18.

[72] Homem de Bittencourt P.I. Jr. and Curi R. Antiproliferative prostaglandins and the MRP/GS-X pump role in cancer immunosuppression and insight into new strategies in cancer gene therapy. Biochemical Pharmacology (2001). , 62(7), 811-19.

[73] Gutierrez, L. L, Maslinkiewicz, A, & Curi, R. and Homem de Bittencourt P.I. Jr. Atherosclerosis: a redox-sensitive lipid imbalance suppressible by cyclopentenone prostaglandins. Biochemical Pharmacology (2008). , 75(12), 2245-62.

[74] Beere, H. M. The stress of dying": the role of heat shock proteins in the regulation of apoptosis. The Journal of Cell Science (2004). Pt 13): 2641-51.

[75] Heck, T. G, & Schöler, C. M. and Homem de Bittencourt P.I. HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? Cell Biochemistry and Function (2011). , 29(3), 215-26.

[76] Ehses, J. A, Boni-schnetzler, M, Faulenbach, M, & Donath, M. Y. Macrophages, cytokines and b-cell death in type 2 diabetes. The Biochemical Society Transactions (2008). Pt 3): 340-2.

[77] Donath, M. Y, Schumann, D. M, Faulenbach, M, Ellingsgaard, H, Perren, A, & Ehses, J. A. Islet inflammation in type 2 diabetes. Diabetes Care (2008). Suppl 2): S, 161-4.

[78] Wang, C, Guan, Y, & Yang, J. Cytokines in the progression of pancreatic β-cell dysfunction. The International Journal of Endocrinology (2010). , 1, 1-10.

[79] Mandrup-poulsen, T. The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia (1996). , 39(9), 1005-29.

[80] Kiely, A, Robinson, A, Mcclenaghan, N. H, Flatt, P. R, & Newsholme, P. Toll-like receptor agonist induced changes in clonal rat BRIN-BD11 β-cell insulin secretion and signal transduction. The Journal of Endocrinology (2009). , 202(3), 365-73.

[81] Coppieters, K. T, Dotta, F, Amirian, N, Campbell, P. D, Kay, T. W, Atkinson, M. A, Roep, B. O, & Von Herrath, M. G. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. The Journal of Experimental Medicine (2012). , 209(1), 51-60.

[82] Bending, D, Zaccone, P, & Cooke, A. Inflammation and type one diabetes. International Immunology (2012). , 24(6), 339-46.

[83] Turley, S, Poirot, L, Hattori, M, Benoist, C, & Mathis, D. Physiological beta-cell death triggers priming of self-reactive T cells by dendritic cells in type-2 diabetes model. The Journal of Experimental Medicine (2003). , 198(10), 1527-37.

[84] Elenkov, I. J, & Chrousos, G. P. Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. Trends in Endocrinology and Metabolism (1999). , 10(9), 359-68.

[85] Homem de Bittencourt Jr, P.I. and Newsholme P. A novel L-arginine/L-glutamine coupling hypothesis: implications for type 1 diabetes, Type 1 Diabetes- Complications, Pathogenesis, and Alternative Treatments, Chih-Pin Liu (Ed.), 978-9-53307-756-7InTech (2011). Available from: http://www.intechopen.com/books/type-1 diabetes-complications-pathogenesis-and-alternative-treatments/a-novel-l-arginine-l-glutamine-coupling-hypothesis-implications-for-type-1-diabetes

[86] Barthson, J, Germano, C. M, Moore, F, Maida, A, Drucker, D. J, Marchetti, P, Gysemans, C, Mathieu, C, Nunez, G, Juriscova, A, Eizirik, D. L, & Gurzov, E. N. Cytokines tumour necrosis factor-(alpha) and interferon-(gamma) induce pancreatic (beta)-cell apoptosis through STAT1-mediated Bim activation. The Journal of Biological Chemistry (2011). , 286(45), 39632-43.

[87] Ehses, J. A, Perren, A, Eppler, E, Ribaux, P, Pospisilik, J. A, Maor-cahn, R, Gueripel, X, Ellingsgaard, H, Schneider, M. K, Biollaz, G, Fontana, A, Reinecke, M, Homo-delarche, F, & Donath, M. Y. Increased cell number of islet associated macrophages in type 2 diabetes. Diabetes (2007). , 56(9), 2356-70.

[88] Boni-schnetzler, M. M. L, Ehses, J. A, Weir, G. C, & Donath, M. Y. (2007). IL-1beta expression is induced by glucose and IL-1beta auto-stimulation, and increased in beta cells of type 2 diabetics. Diabetes 2007; 56(Suppl 1): A413.

[89] Ryan, A, Murphy, M, Godson, C, & Hickey, F. B. Diabetes mellitus and apoptosis: inflammatory cells. Diabetes and Apoptosis (2009). , 14(12), 1435-50.

[90] Donath, M. Y, & Shoelson, S. E. Type 2 diabetes as an inflammatory disease. Nature Reviews Immunology (2011). , 11(2), 98-107.

[91] Pasarica, M, Rood, J, Ravussin, E, Schwarz, J. M, Smith, S. R, & Redman, L. M. Reduced oxygenation in human obese adipose tissue is associated with impaired insulin suppression of lipolysis. The Journal of Clinical Endocrinology and Metabolism (2010). , 95(8), 4052-5.

[92] Weisberg, S. P, Mccann, D, Desai, M, Rosenbaum, M, Leibel, R. L, & Ferrante Jr., A. W. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of Clinical Investigations (2003). , 112(12), 1796-808.

[93] Xu, H, Barnes, G. T, Yang, Q, Tan, G, Yang, D, Chou, C. J, Sole, J, Nichols, A, Ross, J. S, Tartaglia, L. A, & Chen, H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin-resistance. The Journal of Clinical Investigations (2003). , 112(12), 1821-30.

[94] Arkan, M. C, Hevener, A. L, Greten, F. R, Maeda, S, Li, Z. W, Long, J. M, Wynshaw-boris, A, Poli, G, Olefsky, J, & Karin, M. IKK-β links inflammation to obesity-induced insulin-resistance. Nature Medicine (2005). , 11(2), 191-8.

[95] Bowie, A, & O Neill, L.A.J. The interleukin-1 receptor/toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. Journal of Leukocyte Biology (2000).

[96] Nishikori, M. Classical and alternative NFκB activation pathways and their role in lymphoid malignancies. The Journal of Clinical and Experimental Hematopathology (2005). , 45(1), 15-24.

[97] Donath, M. Y, Storling, J, Maedler, K, & Mandrup-poulsen, T. Inflammatory mediators and islet β-cell failure: a link between type 1 and type 2 diabetes. The Journal of Molecular Medicine (Berlin) (2003). , 81(8), 455-70.

[98] Morgan, M. J, & Liu, Z. G. Crosstalk of reactive oxygen species and NFκB signalling. Cell Research (2011). , 21(1), 103-15.

[99] Wullaert, A, Heyninck, K, & Bayaert, R. Mechanisms of crosstalk between TNF-induced NFκB and JNK activation in hepatocytes. Biochemical Pharmacology (2006). , 72(9), 1090-101.

[100] Newsholme, P, Haber, E. P, Hirabara, S. M, Rebelato, E. L. O, Procopio, J, Morgan, D, Oliveira-emilio, H. C, Carpinelli, A. R, & Curi, R. Diabetes associated cell stress and dysfunction: role of mitochondria and non-mitochondrial ROS production and activity. The Journal of Physiology (2007). Pt 1): 9-24.

[101] Schoonbroodt, S, & Piette, J. Oxidative stress interference with nuclear factor-κ B activation pathways. Biochemical Pharmacology (2000). , 60(8), 1075-83.

[102] Shimabukuro, M, Zhou, Y. T, Levi, M, & Unger, R. H. Fatty acid-induced beta-cell apoptosis: a link between obesity and diabetes. Proceedings of the National Academy of Sciences (1998). , 95(5), 2498-502.

[103] Otero, M, Lago, R, Lago, F, Casanueva, F. F, Dieguez, C, Gomez-reino, J. J, & Gualillo, O. Leptin, from fat to inflammation: old questions and new insights. FEBS Letters (2005). , 579(2), 295-301.

[104] Zhang, F, Basinski, M. B, Beals, J. M, Briggs, S. L, Churgay, J. M, & Clawson, D. K. DiMarchi R.D., Furman T.C., Hale J.E., Hsiung H.M., Schoner B.E., Smith D.P., Zhang X.Y., Wery J.P. and Schevitz R.W. Crystal structure of the obese protein leptin-E100. Nature (1997). , 387(6629), 206-9.

[105] Matarese, G, Sanna, V, Lechler, R. I, Sarvet-nick, N, Fontana, S, & Zappacosta, S. and La Cava A. Leptin accelerates autoimmune diabetes in female NOD mice. Diabetes (2002). , 51(5), 1356-61.

[106] Wolf, A. M, Wolf, D, Rumpold, H, Enrich, B, & Tilg, H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochemical and Biophysical Research Communications (2004). , 323(2), 630-5.

[107] Harman-boehm, I, Bluher, M, Redel, H, Sion-vardy, N, Ovadia, S, Avinoach, E, Shai, I, Kloting, N, Stumvoll, M, Bashan, N, & Rudich, A. Macrophage infiltration into omental versus subcutaneous fat across different populations: effects of regional adiposity and the comorbidities of obesity. The Journal of Clinical Endocrinology and Metabolism (2007). , 92(6), 2240-7.

[108] Odegaard, J. I, & Chawla, A. Connecting type 1 and type 2 diabetes through innate immunity. Cold Spring Harbour Perspectives in Medicine (2012). a007724.

[109] Cnop, M, Welsh, N, Jonas, J. C, Jorns, A, Lenzen, S, & Eizirik, D. L. Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: Many differences, few similarities. Diabetes (2005). Suppl 2): S, 97-107.

[110] Lee, S. C, & Pervaiz, S. Apoptosis in the pathophysiology of diabetes mellitus. The International Journal of Biochemistry and Cell Biology (2007). , 39(3), 497-504.

[111] Back, S. H, Kang, S. W, Han, J, & Chung, H. T. Endoplasmic reticulum stress in the β-cell pathogenesis of type 2 diabetes. Experimental Diabetes Research (2012).

[112] Yip, L, Su, L, & Sheng, D. Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. Nature Immunology (2009). , 10(9), 1026-33.

[113] Clark, R, & Griffiths, G. M. Lytic granules, secretory lysosome and disease. Current Opinion in Immunology (2003). , 15(5), 516-21.

[114] Ashkenazi, A, & Dixit, V. M. Death receptors: signalling and modulation. Science (1998). , 281(5381), 1305-8.

[115] Sakamoto, S, & Kyprianou, N. Targeting anoikis-resistance in prostate cancer metastasis. Molecular Aspects of Medicine (2010). , 31(2), 205-14.

[116] Chang, H. Y, & Yang, X. Proteases for cell suicide: functions and regulation of caspases. Microbiology & Molecular Biology Reviews (2007). , 64(4), 821-46.

[117] Batinac, T, Zamolo, G, Coklo, M, & Hadzisejdic, I. Possible key role of granzyme B in keratoacanthoma regression. Medical Hypotheses (2006). , 66(6), 1129-32.

[118] Sutton, V. R, Wowk, M. E, Cancilla, M, & Trapani, J. Caspase activation by granzyme B is indirect, and caspase autoprocessing requires the release of pro-apoptotic mitochondrial factors. Immunity (2003). , 18(3), 319-29.

[119] Li, H, Zhu, H, Xu, C. J, & Yuan, J. Cleavage of Bid by caspase-8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell (1998). , 94(4), 494-501.

[120] Yang, S. H, Chein, C. M, Lu, M. C, Hu, X. W, & Lin, S. R. Up-regulation of Bax and endonuclease G, and down-modulation of Bcl-XL involved in cardiotoxin III-induced apoptosis in K562 cells. Experimental and Molecular Medicine (2006). , 38(4), 435-44.

[121] Green, D. R, & Reed, J. C. Mitochondria and apoptosis. Science (1998). , 281(5381), 1309-12.

[122] Breckenridge, D. G, & Xue, D. Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases. Current Opinion in Cell Biology (2004). , 16(6), 647-52.

[123] Wang, Z. B, Liu, Y. Q, & Cui, Y. F. Pathways to caspase activation. Cell Biology International (2005). , 29(7), 489-96.

[124] Portt, L, Norman, G, Clapp, C, Greenwood, M, & Greenwood, M. T. Anti-apoptosis and cell survival: A review. Biochimica et Biophysica Acta (2011). , 1813(1), 238-59.

[125] Ferri, K. F, & Kroemer, G. Organelle-specific initiation of cell death pathways. Nature Cell Biology (2001). E, 255-63.

[126] Brandt, B, Abou-eladab, E. F, Tiedge, M, & Walzel, H. Role of the JNK/c-Jun/AP-1 signalling pathway in galectin-induced T-cell death. Cell Death and Disease (2010). e23., 1.

[127] Rahier, J, Guiot, Y, Goebbels, R. M, Sempoux, C, & Henquin, J. C. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, Obesity and Metabolism (2008). Suppl 4): 32-42.

[128] Chang-chen, K. J, Mullur, R, & Bernal-mizrachi, E. cell failure as a complication of diabetes. Reviews in Endocrine Metabolic Disorders (2008). , 9(4), 329-43.

[129] Gehrmann, W, Elsner, M, & Lenzen, S. Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic β-cells. Diabetes, Obesity and Metabolism (2010). Suppl 2): 149-58.

[130] Chakrabarti, A, Chen, A. W, & Varner, J. D. A review of the mammalian unfolded protein response. Biotechnology and Bioengineering (2011). , 108(12), 2777-93.

[131] Kaufman, R. J Stress signalling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes & Development (1999). , 13(10), 1211-33.

[132] Szegezedi, E, Logue, S. E, Gorman, A. M, & Samali, A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Reports (2006). , 7(9), 880-5.

[133] Nakagawa, T, & Yuan, J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. Journal of Cell Biology (2000). , 150(4), 887-94.

[134] Rao, R. V, Peel, A, & Loqvinova, A. del Rio G., Hermel E., Yokota T., Goldsmith P.C., Ellerby L.M., Ellerby H.M. and Bredesen D.E. Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Letters (2002).

[135] Putcha, G. V, Le, S, Frank, S, Besirli, C. G, Clark, K, Chu, B, Alix, S, & Youle, R. J. LaMarche A., Maroney A.C. and Johnson E.M. Jr. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. Neuron (2003). , 38(6), 899-914.

[136] Donath, M. Y, Ehses, J. A, Maedler, K, Schumann, D. M, Ellingsgaard, H, Eppler, E, & Reinecke, M. Mechanisms of β-cell death in type 2 diabetes. Diabetes (2005). Suppl 2): S, 108-13.

[137] Cunha, D. A, Hekerman, P, Ladriere, L, Bazarra-castro, A, Ortis, F, Wakeham, M. C, Moore, F, Rasschaert, J, Cardozo, A. K, Bellomo, E, Overbergh, L, Mathieu, C, Lupi, R, Hai, T, Herchuelz, A, Marchetti, P, Rutter, G. A, Eizirik, D. L, & Cnop, M. Initiation and execution of lipotoxic ER stress in pancreatic β-cells. The Journal of Cell Science (2008). Pt 14): 2308-18.

[138] Cnop, M. Fatty acids and glucolipotoxicity in the pathogenesis of type 2 diabetes. The Biochemical Society Transactions (2008). Pt 3): 1180-6.

[139] Haataja, L, Gurlo, T, Huang, C. J, & Butler, P. C. Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocrine Reviews (2008). , 29(3), 303-16.

[140] Larsen, C. M, Faulenbach, M, Vaag, A, Volund, A, Ehses, J. A, Seifert, B, Mandrup-poulsen, T, & Donath, M. Y. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. New England Journal of Medicine (2007). , 356(15), 1517-26.

[141] Mastrandrea, L, Yu, J, Behrens, T, Buchlis, J, Albini, C, Fourtner, S, & Quattrin, T. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomised, placebo-controlled, double blind study. Diabetes Care (2009). , 32(7), 1244-9.

[142] Chung, J, Nguyen, A. K, Henstridge, D. C, Holmes, A. G, Chan, M. H, Mesa, J. L, Lancaster, G. I, Southgate, R. J, Bruce, C. R, Duffy, S. J, Horvath, I, Mestril, R, Watt, M. J, Hooper, P. L, Kingwell, B. A, Vigh, L, Hevener, A, & Febbraio, M. A. HSP72 protects against obesity-induced insulin-resistance. Proceedings of the National Academy of Sciences (2008). , 105(5), 1739-44.

[143] Kurucz, I, Morva, A, Vaag, A, Eriksson, K. F, Huang, X, Groop, L, & Koranyi, L. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin-resistance. Diabetes (2002). , 51(4), 1102-9.

[144] Rodrigues-krause, J, Krause, M, Hagan, O, De Vito, C, Boreham, G, Murphy, C, Newsholme, C, & Colleran, P. G. Divergence of intracellular and extracellular HSP72 in type 2 diabetes: does fat matter? Cell Stress Chaperones. (2012). , 17(3), 293-302.

[145] Gupte, A. A, Bomhoff, G. L, Swerdlow, R. H, & Geiger, P. C. Heat treatment improves glucose tolerance and prevents skeletal muscle insulin-resistance in rats fed a high-fat diet. Diabetes (2009). , 58(3), 567-78.

http://dx.doi.org/10.5772/55349

[146] Krause, M. S. and Homem de Bittencourt P.I. Jr. Type 1 diabetes: can exercise impair the autoimmune event? The L-arginine/glutamine coupling hypothesis. Cell Biochemistry and Function (2008). , 26(4), 406-33.

[147] Mandrup-poulsen, T, Pickersgill, L, & Donath, M. Y. (2010). Blockade of interleukin-1 in type 1 diabetes. Nature Reviews Endocrinology 2010; , 6(3), 158-66.

[148] Patterson, C. C, Dahlquist, G. G, Gyurus, E, Green, A, & Soltesz, G. and EURODIAB Study Group. Incidence t rends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet (2009). , 373(9680), 2027-33.

INTECH