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Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes

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Abstract

Pancreatic β -cell dysfunction plays an important role in the pathogenesis of both type 1 and type 2 diabetes. Insulin, which is produced in β -cells, is a critical regulator of metabolism. Insulin is synthesized as preproinsulin and processed to proinsulin. Proinsulin is then converted to insulin and C-peptide and stored in secretory granules awaiting release on demand. Insulin synthesis is regulated at both the transcriptional and translational level. The cis-acting sequences within the 5' flanking region and trans-activators including paired box gene 6 (PAX6), pancreatic and duodenal homeobox-1 (PDX-1), MafA, and B-2/Neurogenic differentiation 1 (NeuroD1) regulate insulin transcription, while the stability of preproinsulin mRNA and its untranslated regions control protein translation. Insulin secretion involves a sequence of events in β -cells that lead to fusion of secretory granules with the plasma membrane. Insulin is secreted primarily in response to glucose, while other nutrients such as free fatty acids and amino acids can augment glucose-induced insulin secretion. In addition, various hormones, such as melatonin, estrogen, leptin, growth hormone, and glucagon like peptide-1 also regulate insulin secretion. Thus, the β -cell is a metabolic hub in the body, connecting nutrient metabolism and the endocrine system. Although an increase in intracellular $[Ca^{2+}]$ is the primary insulin secretory signal, cAMP signaling-dependent mechanisms are also critical in the regulation of insulin secretion. This article reviews current knowledge on how β -cells synthesize and secrete insulin. In addition, this review presents evidence that genetic and environmental factors can lead to hyperglycemia, dyslipidemia, inflammation, and autoimmunity, resulting in β -cell dysfunction, thereby triggering the pathogenesis of diabetes.

Keywords

β -cell; diabetes; glucose; hormones; insulin secretion; insulin synthesis

INSULIN

Insulin structure

The crystal structure of insulin is well documented as well as the structural features that confer receptor binding affinity and activity. This has been extensively reviewed and readers are encouraged to visit [1] and [2] for excellent discussions on insulin structure and structure-activity relationships. As discussed in this review, insulin receptor downstream signaling intersects with the signaling pathways of other growth factors, including IGF1 and

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CONFLICTS OF INTEREST

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IGF2 [3]. This demonstrates the importance of identifying receptor ligand agonists as potential insulin-mimetic therapeutic agents in diabetes. This section of the review will focus on the native structure of insulin. For an excellent review on the insulin receptor structure and binding domains, readers are encouraged to visit several references [2, 3].

The 3-D structure of monomeric insulin was first discovered by x-ray crystallography and reported in 1926 [4]. More than 40 years later, the structure of the zinc-containing hexameric insulin was solved [5–8]. 2D NMR studies have also contributed to knowledge on the monomeric, dimeric and hexameric conformations of insulin, all revealing information on the native structure of insulin and the amino acids that confer binding specificity to the insulin receptor [1]. Insulin concentration and surrounding pH influence the conformational state of insulin. The monomers tend to form dimers as the concentration of insulin rises, and in the presence of zinc and favorable pH (10 mM Zn^{++} , pH ~6.0) the monomers assemble into higher order conformations called hexamers [9]. As discussed below, interactions among hydrophobic amino acids in insulin dimer structures favor aggregation as concentrations rise. Once the hexamers are secreted from the β -cell and diffuse into the blood down their concentration gradient, a combination of electrostatic repulsion and decreased concentration of insulin favors the dissociation of insulin into its monomeric form [1]. Hence, the monomer is the active form of insulin, while the hexamer is the storage form of insulin.

The monomeric insulin consists of the 21 amino acid residue “A” chain and 30 amino acid residue “B” chain bound by disulfide linkages. The monomer consists of three disulfide linkages, including two between the A and B chains (A7-B7, A20-B19) and one within the A chain (A7-A11) [4]. The secondary structure of the A chain contains two antiparallel α -helices, formed between the residues A2-A8 and A13 to A19 [1]. These two helices are connected by residues A9 to A12. This conformation brings the two ends of the A chain into close proximity, allowing them to exist side by side.

The secondary structure of the B chain contains both alpha helices and β -sheets [1]. The B chain may exist in two different conformations when crystallized [8]. In the T-state, there is a central alpha helix from B9 to B19 (1→5 helix hydrogen bonding pattern). There is a 1→4 β turn from B20-B23, with the Gly20 and Gly23 residues allowing the chain to fold into a “V” shape. Residues B24 to B30 form an extended β strand structure; the β -turn allows the chain to be in close enough proximity to form a β -sheet with PheB24 and TyrB26 in contact with leucines B11 and B15 of the B-chain alpha helix. In the R state, there is a continuous alpha helix from B1-B19. The disulfide bonds between Cys residues A7-B7 and A20-B19 contribute to the stability of the native insulin structure. The secondary structure of both the A and B chains is surprisingly complex for such small peptides, and these intricate side-chain interactions determine insulin receptor affinity.

The overall tertiary structure of the insulin monomer (A and B chain joined by disulfide bonds) is highly organized and stabilized by specific amino acid side chain interactions. These interactions influence ligand-receptor binding kinetics [1]. The residues A6-A11 and Leu A11, B1 and B15, Ile A2, Phe B24, Val A3, Ile A13, Val B18 and Val B12 comprise the hydrophobic core of the monomeric protein structure. The latter AA residues on the B chain also serve to stabilize the β -turn of the B chain (B20-B23) allowing the β sheet (B23-B30) to fold against the helix and hydrophobic residues. These nonpolar amino acid residues become buried when the monomers form dimers.

At micromolar concentrations insulin monomers form dimers [2]. The insulin dimer is maintained by the antiparallel β sheets at the carboxy terminus of the B chains on each monomer. These β sheets are exposed to the surface of the dimer structure. The interface of

the dimer contains the nonpolar residues discussed above that contribute to the hydrophobic core. Solvent exclusion as a result of interactions among hydrophobic AA residues leads to aggregation, favoring the formation of a higher order structure.

Insulin stored in β -cells is packed into densely clustered “granules” consisting of insoluble crystalline hexameric insulin. The concentration of insulin in these granules is roughly 40 mM [2]. The hexameric form of insulin consists of 6 molecules of insulin peptide arranged as 3 dimers. Although each monomer in the dimer consists of the same peptide sequence, there are some differences in side chain spatial conformation such that there is not perfect 2-fold symmetry [1]. One example is Phe (B-chain, residue 25), which is oriented towards the hydrophobic core of the peptide on one side of the dimer, and folded away from the peptide on the other side of the dimer. The insulin hexamer coordinates two zinc atoms with the imidazole groups of three histidine residues (B chain, residue 10) and three water molecules. In the 2 Zn crystalline structure, all six monomers are in the T conformation discussed above [2]. In the presence of high chloride concentrations in the β -cell, a 4 Zn hexameric structure forms, whereby three of the monomers exist in the R conformation and three in the T form [2]. The R form predominates in phenol-containing crystals [10]. From a pharmacological standpoint, these are important observations, as phenol serves as an antimicrobial and chloride as an isotonic agent in insulin formulations [2]. Interactions between the dimers in the hexamer are weaker than interactions within the dimer, with greater vander Waals separations among the dimers in the hexamer as compared with separations among the monomers in the dimer [1]. Hence, the hexamer configuration is less stable and subject to dissociation when concentrations of insulin fluctuate (e.g., secretion into bloodstream).

Insulin structure-function relationships

Solving the 3D structure of insulin allowed for prediction of regions important in insulin activity. For such a small protein, the secondary and tertiary structures are complex and impressive. As reviewed by Pittman et al [1], the residues that are evolutionarily conserved and important for conformational flexibility are likely those important for determining insulin receptor binding affinity.

There are several regions of the insulin monomer that are important for facilitating receptor binding. The importance of these amino acid residues was discovered by screening insulin analogs with various substitutions and deletions for their ability to activate the insulin receptor. Recombinant DNA technology, allowing for production of active insulin, allowed for development of high-throughput screening by site-directed mutagenesis [2]. These studies revealed several evolutionarily conserved amino acids near the surface of the insulin monomer [5]. Among these, several are located at the amino terminus of the A chain (GlyA1, IleA2, ValA3, GluA4), the carboxy terminus of the A chain (TyrA19, CysA20, AsnA21) and the carboxy terminus of the B chain (glyB23, PheB24, PheB25, TyrB26) [11]. Several of these residues (A21, B23–25) through negative cooperativity, define the “cooperative” site on the insulin structure [2]. The IleA2 and ValA3 are not exposed to the surface of the native insulin structure, but upon displacement of the B chain carboxy terminus during receptor binding become exposed to the surface [12, 13]. Several mutations were identified in these regions that reduce the affinity to the insulin receptor, including LeuB25 for PheB25 (insulin Chicago), SerB24 for PheB24 (insulin Los Angeles) and LeuA3 for ValA3 (insulin Wakayama). Patients harboring these mutations display glucose intolerance and hyperinsulinemia [14–19].

The A chain contains several regions that are important for receptor binding. The amino terminus, when N-acetylated, shows reduced receptor binding by 30 %, indicating that a free positively charged amino terminus is critical for receptor binding [20]. Deleting Gly1 reduces activity by 15 %, indicating that the salt bridge formed between Gly1 and the

carboxy terminus of the B chain is important for correct positioning of the peptide [6]. Additionally, the carboxy terminus of the A chain, particularly TyrA19, CysA20 and AsnA21 are considered to be important for insulin receptor affinity.

The B chain is the most studied in the context of structure-activity relationships, in particular the carboxy terminal domain. The first four residues of the B chain (amino terminus) can be deleted with only modest reductions in receptor binding activity (roughly 70 % activity retained) [21, 22], however; deletion of HisB5 leads to a major reduction in activity (15 % activity retained). Furthermore, LeuB6 is critical for binding activity and deletion results in a mutant with less than 1 % binding affinity [23]. CysB7 is also important, with obvious importance in maintaining the disulfide linkage between the A and B chains. HisB10 is key for maximal insulin activity and when substituted for AspB10, proinsulin is not processed to insulin, leading to increases in circulating proinsulin [24, 25]. Interestingly though, synthetic insulin containing the AspB10 substitution shows a 500 % increase in binding affinity as compared with the “wild type” synthetic insulin [26]. The B chain carboxy terminus contains evolutionarily conserved residues important for receptor binding, including GlyB23, PheB24, PheB25 and TyrB26. Specifically, PheB24 forms hydrogen bonds that are critical for dimer formation and PheB25 shows different conformations on the two molecules that comprise the dimer, indicating that it is important for conformation of the native insulin structure [6].

In summary, while numerous amino acid residues are important for insulin binding to the insulin receptor, namely N-terminal A chain residues and C-terminal B chain residues, it is likely that conformational changes in the insulin secondary and tertiary structure dictate the stability of the protein and affinity for the insulin receptor. The exact mechanism whereby insulin binds to its receptor has yet to be elucidated. Hence, a more detailed structure of the insulin-receptor complex combined with functional activity assays should lead to a greater understanding of the factors that determine receptor-ligand binding affinity and specificity.

PANCREATIC β -CELL PHYSIOLOGY

Insulin biosynthesis

The secreted insulin consists of 51 amino acids with a molecular weight of 5.8 kDa. However, the *insulin* gene encodes a 110-amino acid precursor known as preproinsulin. As with other secreted proteins, preproinsulin contains a hydrophobic N-terminal signal peptide, which interacts with cytosolic ribonucleoprotein signal recognition particles (SRP) [27]. SRP facilitates preproinsulin translocation across the rough endoplasmic reticulum (rER) membrane into the lumen. This process occurs via the peptide-conducting channel [28, 29], where the signal peptide from preproinsulin is cleaved by a signal peptidase to yield proinsulin [30]. Proinsulin then undergoes folding and formation of three disulfide bonds [31], a process requiring a diverse range of endoplasmic reticulum (ER) chaperone proteins such as the protein-thiol reductase [32]. Subsequent to maturation of the three dimensional conformation, the folded proinsulin is transported from the ER to the Golgi apparatus where proinsulin enters immature secretory vesicles and is cleaved to yield insulin and C-peptide. Insulin and C-peptide are then stored in these secretory granules together with islet amyloid polypeptide (IAPP or amylin) and other less abundant β -cell secretory products [33, 34].

Although insulin biosynthesis is controlled by multiple factors, glucose metabolism is the most important physiological event that stimulates insulin gene transcription and mRNA translation [35]. In 3-day fasted rats, glucose injection increased relative proinsulin mRNA levels by three- to four-fold within 24 h and this effect was blocked by pharmacological inhibition of transcription with actinomycin D [36]. These results suggest that glucose plays

a central role in regulation of insulin biosynthesis which is controlled at least partially via alterations in proinsulin mRNA expression. In addition, glucose is an important factor for maintaining insulin mRNA stability. Results from in vitro studies demonstrated that insulin mRNA stability was reduced under lower glucose concentrations and increased under higher glucose concentrations [37, 38]. Interestingly, elevation of intracellular cAMP levels can prevent this reduction [39].

Most animals have only a single copy of the insulin gene, but rodents have two non-allelic insulin genes (insulin I and II). They differ in their number of introns and chromosomal locations [40]. In all insulin genes the 5'-flanking region determines its tissue- and cell-type-specific expression [41]. The transcriptional factor binding sites that determine insulin's exclusive expression in β -cells are located between -520 and +1 base pairs (bp) relative to the transcription start site (TSS) in both rat and human insulin genes [35, 41, 42]. Among mammalian insulin genes, there is a conserved sequence located from -350 bp to the TSS, which controls cell-type-specific expression of insulin. Most transcriptional regulation occurs through interactions within these conserved sequences. Studies have shown that the sequence between -340 and +91 is the major insulin gene transcription enhancer region, which determines cell-specific and glucose-regulated insulin gene expression [43–47].

Regulation of insulin transcription

Insulin biosynthesis is regulated both at transcriptional and translational levels. In a mouse β -cell, there are roughly 13,000 insulin granules. They occupy more than 10% of the total cell volume [48]. Each granule contains approximately 200,000 insulin molecules [49]. However, insulin content in β -cells is highly dynamic. Insulin accumulates in the presence of nutrients and decreases in response to nutrient deprivation. The ability of β -cells to quickly respond to cellular signals is generally due to transcriptional regulation. A number of discrete sequence elements within the promoter region of insulin gene, named A, C, E, Z, and CRE elements determine localization of insulin in β -cells and also serve as binding sites for several β -cell transcription factors to regulate insulin gene expression [50]. The transcription factor binding sites that are located within a region spanning ~400 base pairs (bp) relative to the TSS are determinants of β -cell-specific expression of insulin [50].

A number of cis- and trans- transcriptional factors are associated with the activation of the insulin enhancer region. In all characterized insulin enhancer sequences the A, C, and E elements are contained in core binding motifs [51].

A elements—The A elements are multiple A/T rich elements located in the conserved control region of the insulin gene [52]. There is a TAAT core in each of these A elements that serves as the central DNA binding recognition motif for homeodomain proteins [53, 54], including duodenal homeobox-1(PDX-1) [55–57], Cdx2/3 [58], and Isl-1 [59]. PDX-1 is the predominant binding factor detected with insulin A element probes in pancreatic β -cell extracts [55, 60, 61]. This factor was first characterized as an insulin [55, 60–62] and somatostatin [43,44] transcriptional factor. The expression of PDX-1 in adult pancreas is generally restricted to islet β -cells (~91%). Only a small subset of δ -cells (~15%) express PDX-1 and levels in exocrine acinar cells are extremely low [57, 62–64]. The Cdx2/3, while expressed in β -cells and α -cells, appears to play a less important role in islet function, because Cdx2/3 mutant mice have defects in only intestinal function [65]. The Isl-1 is present in all types of islet cells [66] and can activate somatostatin [67], glucagon [68], and IAPP [69] gene expression. It also plays an essential role in islet formation during embryo development [70].

C element—There are two C elements in the insulin gene promoter. The C1 element is located between 118 and 107 bp upstream of the rat insulin TSS [71]. Rat insulin promoter element 3b (RIPE3b)1 and RIPE3b2 [71, 72] are two factors that form protein-DNA complexes within the C1 element. RIPE3b2 consists of the p58, p62, and p110 subunits [73]. RIPE3b2 does not contribute to β -cell-specific expression of the insulin gene [71, 73], and RIPE3b2-binding activity is present in a variety of other tissues [71]. The DNA-binding component of the RIPE3b1 was recently identified as MafA [74–76], which is expressed exclusively in β -cells [77]. MafA also mediates glucose-regulated and fatty acid-inhibited insulin expression. Prolonged exposure of islets to fatty acid and high glucose inhibits insulin gene transcription by impairing nuclear cellular expression of MafA. [78–80]. MafA deficient animals showed no defects in β -cell development, although impaired insulin expression in adult islets was observed [81].

The C2 element, which is located at –317/–311 bp in the rat I insulin gene was termed the pancreatic islet cell enhancer sequence (PISCES). It was found to contribute to insulin, glucagon, and somatostatin transcription in α -, β -, and ϵ -cells, respectively [82]. Furthermore, PISCES is a binding site for PAX6 both in insulin and glucagon genes [83]. Like other PAX transcriptional factors, PAX6 contains a paired box bipartite DNA binding domain. PAX6 is required for normal transcription of insulin genes and islet development [83]. Besides PAX6, PAX4 is also a paired/homeodomain protein expressed in the pancreas. Although both PAX4 and PAX6 can bind to PISCES [84], PAX4 is only detected transiently in β -cells during early development and absent in adult β -cells [85]. PAX4 is reported to suppress PAX6-induced trans-activation; however it is unclear if PAX4 regulates insulin expression in vivo because of its narrow window of expression.

E element—The E elements (5'-GCCATCTG-3') are two separated mini-enhancer units within the insulin enhancer [43, 71, 86]. Rodents have two E elements (–241 to –233 bp and –112 to –104 bp) in the insulin I gene; while other mammals have only one (approximately –100 to –91 bp) [51]. The core insulin E element (5'-CANNTG-3') is also found in the heavy-chain immunoglobulin and muscle creatine kinase control elements [87–89]. The factors that bind the E element contain a helix-loop-helix domain (HLH) that is important in facilitating protein-protein interactions, and a contiguous amino terminal basic region (b) that is necessary for DNA-protein binding. This motif is shared by a number of transcriptional factors required in cell type determination including the muscle determination proteins MyoD [90], Myf-5 [91], myogenin [92], and the proteins of the drosophila achaete-scute complex, which are important in neural development [93]. The E element activators include BETA2/NeuroD1, E2/5, E12 and E47. BETA2/NeuroD1 is enriched in islets [94, 95], while E2/5, E47 [71, 96, 97], and E12 [98] are widely distributed. BETA2/NeuroD1 is important in regulating insulin gene expression and β -cell survival, and the endocrine pancreas-specific deficiency of BETA2/NeuroD in mice causes massive β -cell apoptosis and subsequent diabetes and early death [99, 100].

Z element—The Z element is located upstream of the A element (–292 to –243 bp) and is unique to human insulin. A glucose-sensitive DNA-binding complex termed ZaI binds to the region of –287 to –271 bp within the Z element in primary islet cells [101]. Z element also functions as a transcriptional repressor in transformed β -cell lines and primary fibroblast cells [101, 102]. Recent studies show that A element activation depends on the present of the Z element [103]. PDX-1 and MafA regulate insulin gene transcription through activation of the Z element [104].

Cyclic AMP response element (CRE)—The human insulin gene promoter contains four CRE sites: CRE1 at –210 bp, CRE2 at –183 bp, CRE3 at +18 bp, and CRE4 at +61 bp

[105]; within the core of each CRE there is a sequence similar to the CRE consensus sequence [106]. A variety of transcription factors can regulate insulin gene transcription by binding to the consensus CRE sequence of 5'-TGACGTCA-3' [106]. These transcriptional factors are members of the CRE binding protein (CREB)/ATF family [107]. The CREB/ATF family of transcription factors are basic region leucine zipper (bZIP) proteins that share a common cluster of basic amino acids at the N-terminus of the bZIP domain, which binds to the CRE site to initiate insulin transcription [108].

Modes of gene regulation can be species-specific, and thus interpretation of data from animal models and extrapolation to humans must be exercised with caution. For example, hepatocyte nuclear factor (HNF)-1 [109] and Isl-1 [110] can bind to the A elements to stimulate rat insulin-I gene transcription. In addition, Cdx-3 [58] and HMGI(Y) [111] bind specifically to the A3/A4 element, which is unique to rat insulin-I. Besides regulation at the gene promoter regions as described above, control of ER load, granule counting, and cell cooperation provide essential feedback loops for controlling insulin transcription [112], which will be further elaborated upon in this review.

Regulation of insulin translation

In response to nutrients, β -cells enhance their overall speed of protein translation, which is at least partly controlled by dephosphorylation of eukaryotic initiation factor 2a (eIF2a) via protein phosphatase 1 (PP1) [113]. For example, exposure of β -cells to high glucose for 2 hours significantly decreases the ratio of phosphorylated eIF2a to eIF2a [114]. However, there are additional mechanisms to regulate glucose-induced insulin translation, since the overall protein translation induced by glucose compared to the fasting state in β -cells was only 3-fold compared with an up to 8-fold induction in proinsulin translation [115].

The pancreatic ER kinase (PERK) plays an important role in regulating translational events. It phosphorylates eIF2a [116], thereby regulating insulin translation [117]. PERK phosphorylation of eIF2a can be partially compensated for by other kinases [118, 119]. PERK mutation results in Wolcott-Rallison syndrome associated with permanent neonatal diabetes in humans [120]. PERK-deficient mice not only develop severe defects in insulin synthesis, but also in β -cell proliferation and differentiation, leading to permanent neonatal diabetes as seen in humans. Using pancreas and β -cell specific knockout mouse models, it was determined that while PERK is required during the fetal and neonatal stages for proper development of β -cell mass, it is not required in adults for maintaining β -cell mass [121]. The Wolfram Syndrome gene *WSF1*, taking its name from the Wolfram syndrome, is unregulated by ER stress via Inositol-Requiring Protein 1 (IRE1)- α and PERK [122, 123]. At the beginning of glucose exposure, IRE1 stimulates insulin synthesis via *WFS1*, while after prolonged exposure, it may reduce insulin production via X-box-binding protein 1 (XBP1) [124]. The β -cells have evolved a mechanism to detect the amount of insulin stored and secreted and adjust insulin synthesis accordingly. A granule transmembrane protein called islet cell autoantigen 512 (ICA512), is a crucial part of this feedback control. Insulin granules travel a long distance on tubulin tracks before arriving at the peripheral actin network [125]. Before becoming linked to the cytoskeleton, insulin granules are anchored to actin cortex via ICA512 and β 2-syntrophin [126]. Upon activation, the granule membrane fuses transiently to the cell membrane to release insulin. Elevated Ca^{2+} levels in the meantime activate the protease μ -calpain to cleave away a cytosolic fragment from ICA512. The free ICA512 cytosolic fragment then moves to the nucleus and binds to the tyrosine-phosphorylated transcriptional factor STAT5 to prevent STAT 5 from dephosphorylation, which in turn upregulates insulin transcription [127]. Nuclear free ICA512 cytosolic fragments also bind to sumoylating enzyme PIAS γ . The sumoylation of ICA512 by PIAS γ reverses the binding of ICA512 to STAT5 [127]. Hence, the release of insulin from secretory granules is communicated to the nucleus, which serves as a positive feedback

mechanism to initiate insulin translation for maintaining an adequate amount of stored insulin.

In addition to transcriptional regulation, β -cells are also able to adjust insulin production in response to immediate environmental triggers by regulating the speed of insulin translation. For example, exposure of rat islets to 25 mM glucose for 1 hour led to an induction in intracellular proinsulin levels by up to ten-fold from baseline (2.8 mM glucose), whereas proinsulin mRNA quantities remained the same [128]. Results from an earlier study demonstrated that this acute glucose-stimulated insulin synthesis is independent of mRNA synthesis within the first 45 min because blockage of transcription only slowed insulin accumulation after that time frame [129]. In addition, insulin mRNA stability, which depends on nutrient status, is an important factor that influences insulin protein synthesis [36]. Results of in vitro studies showed that insulin mRNA stability decreases under lower glucose concentrations and increases under high glucose conditions [37, 38]. In the absence of glucose, insulin mRNA levels in β -cells decrease sharply, which is reversed by elevation of intracellular cAMP levels [39]. The same phenomena were observed in animal studies. Rats fasted for 3 days have only 15–20% of the levels of pancreatic insulin mRNA that were measured in the control animals [36]. Thus, post-transcriptional regulation controls the modulation of immediate insulin synthesis, while regulation at the transcriptional level contributes to the modulation of delayed insulin synthesis.

Polypyrimidine tract binding proteins (PTBPs) are proteins that regulate mRNA translation. They are involved in exon repression while mRNA is undergoing splicing in nuclei and stabilization and ribosome recruitment in the cytosol [130–132]. They upregulate translation both by extending mRNA viability and by stimulating the initiation of translation. Cytosolic PTBP1 binds to a CU-rich sequence in the 3' UTR of proinsulin, which stabilizes proinsulin mRNA [130–132]. PTBP1 also upregulates translation of several insulin granule proteins. PTBP1 binding to ICA512 mRNA decreases 3' UTR decay. Deletion of the PTB binding site substantially reduced prohormone convertase 2 (PC2) translation. Insulin and insulin granule protein mRNA share a similar affinity to the RNA binding protein PTBP1, which enables their gene-specific activation by glucose. It was recently found that there is a conserved region (40–48nt) from the 5' UTR of proinsulin mRNA that plays an essential role in glucose regulation of proinsulin translation, because removal of this region blocked glucose-stimulated proinsulin translation [115].

Regulation of insulin secretion

Insulin is an important hormone required for normal metabolism. In healthy subjects, insulin release is exquisitely exact to meet the metabolic demand. Specifically, β -cells sense changes in plasma glucose concentration and respond by releasing corresponding amounts of insulin [133]. To sense the nutritional state, β -cells are clustered in islets that strategically connect to the vasculature. Islets form a dense network with small blood vessels and receive 10 times the amount of blood than cells in the surrounding exocrine regions. Capillaries surrounding islets show a remarkable number of small pores called fenestrae that allow for a greater nutrient exchange between the circulation and surrounding tissues. This structure enhances permeability, allowing unrestricted nutrient access so that β -cells can sense the nutritional state quickly. Fenestrations also permit rapid insulin diffusion into the blood [112]. In addition to glucose, some amino acids and fatty acids also regulate insulin secretion. A schematic illustration of nutrient regulated insulin secretion is shown in Fig. (1).

Glucose and insulin secretion—The β -cells respond to many nutrients in the blood circulation, including glucose, other monosaccharides, amino acids, and fatty acids. Glucose

is evolutionarily the primary stimuli for insulin release in some animal species, because it is a principal food component and can accumulate immediately after food ingestion. Indeed, in rodents and humans, the amplitude of insulin secretion induced by glucose is much larger compared with that stimulated by protein or fat. Oral ingestion of 75 g of glucose will cause plasma insulin to rise from a basal level (20–30 pmol/L) to 250–300 pmol/L in 30 min, while intake of a similar amount of fat or a fat plus protein diet will only increase plasma insulin levels to 50 and 60 pmol/L, respectively, in human subjects [134]. While glucose is the obligate fuel source for neurons [112], other cells, including β -cells can use alternative fuel sources during brief periods of starvation, an adaptation that could predispose them to glucolipotoxicity, as discussed later in this review.

β -cells do not appear to contain membrane-bound glucose receptors but are equipped with several sensing devices that measure circulating glucose. Glucose transporter 2 (GLUT2), constitutively expressed in β -cells, is the first encountered glucose sensor in β -cells. Glucose equilibrates in β -cells via GLUT2-mediated facilitated diffusion. GLUT2 is the only glucose transporter expressed in β -cells. It is also expressed in the liver, and to a lesser extent, in renal and intestinal absorptive cells. Unlike GLUT4, which is primarily expressed in muscle and fat cells, mobilization of GLUT2 to the plasma membrane is insulin-independent and the transporter protein shows a low substrate affinity, ensuring high glucose influx. After entering β -cells, glucose is phosphorylated by the rate-limiting enzyme glucokinase, a subtype of hexokinase. Glucokinase is expressed in only four types of mammalian cells: hepatic cells, β -cells, enterocytes, and glucose-sensitive neurons [112]. Two important properties enable glucokinase to function as a glucose sensor in β -cells, distinguishing it from other hexokinases. The first property is its relatively lower affinity for glucose than other hexokinases. Its K_m is only 6 mmol/L, falling in the middle of the normal blood glucose range (4–10 mmol/L), while other hexokinases function at maximal velocity at this glucose concentration. The second property is that it is not inhibited by its product, often a regulatory feature in metabolism. This feature enables its continued activity in spite of a high glycolysis load. Glucokinase is thus the rate-limiting step in β -cell glucose metabolism and it is considered to be an important glucose sensor [112].

The endpoint of glycolysis is the metabolic substrate pyruvate that is then oxidized through the tricarboxylic acid *cycle* by mitochondria in β -cells to produce ATP. In other types of cells, pyruvate can be converted to lactate by pyruvate dehydrogenase. However, because β -cells lack this enzyme, pyruvate is mainly metabolized to produce metabolic coupling factors through 2 routes: 1) After being metabolized to acetyl-coA it enters glucose oxidation and 2) anaplerosis. Pyruvate oxidation through the tricarboxylic acid *cycle* (TCA) by mitochondria is the major signaling pathway coupled to “ATP-sensitive potassium (K_{ATP}) channel-dependent insulin release”, which increases the intracellular ATP/ADP ratio, sequentially leading to closure of K_{ATP} channels, depolarization of the plasma membrane, opening of voltage-dependent Ca^{2+} channels, influx of Ca^{2+} , and eventual activation of exocytosis of insulin-containing granules. Anaplerosis serves to replenish the carbon pool in the TCA cycle. After the cycle is filled with intermediates, these carbons can exit via cataplerosis. Some products derived in these processes can act as insulin secretion signals, which include NADPH, malonyl-CoA, and glutamate. These molecules reportedly amplify K_{ATP} channel-dependent insulin secretion [134, 135].

A third glucose signal results from the formation of glycerol-3-phosphate (Gly3P). After glucose is phosphorylated into glucose-6-phosphate (G6P) by glucokinase, G6P can enter glycolysis to generate pyruvate. It can also be metabolized into dihydroxyacetone phosphate (DHAP) part of the way through the pathway to provide Gly3P. Gly3P is important for generating lipid metabolic coupling factors such as long-chain acyl-CoA and diacylglycerol (DAG) that can augment insulin secretion. Gly3P/DAG is an alternative pathway that is

independent of mitochondrial metabolism of glucose to produce metabolic coupling factors to stimulate insulin release. Gly3P can also replenish NAD⁺ to promote β -cell glycolysis via the mitochondrial Gly3P NADH shuttle process, which then activates mitochondrial energy metabolism and triggers insulin secretion [136, 137].

Amino acids and insulin secretion—Individual amino acids at physiological concentrations are poor insulin secretagogues. However, certain combinations of amino acids at physiological concentrations or higher can augment GSIS [138]. For example, glutamine alone does not stimulate insulin secretion or enhance GSIS, but a combination of glutamine with leucine can enhance GSIS from β -cells [139]. Leucine can activate glutamate dehydrogenase, which converts glutamate to α -ketoglutarate. Glutamine, after converted into glutamate by glutaminase in the cytosol, can enter the TCA cycle via α -ketoglutarate, which results in ATP production, thereby enhancing insulin secretion [138]. Without leucine, glutamine is metabolized to γ -aminobutyric acid (GABA) and aspartate. Moreover, some amino acids can indirectly influence β -cell insulin secretion. During the fasting period, proteins in skeletal muscle are catabolized and amino acids are subsequently metabolized for generating energy. Free amino acids, including alanine and glutamine, are released into the blood and serve as potent glucagon secretagogues. This results in elevation of blood glucose levels, which then triggers insulin secretion. Dietary amino acids can also induce insulin secretion via incretin-dependent mechanisms. Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two major incretin hormones secreted from the gastrointestinal tract. Ingestion of nutrients in the gut, including glucose and amino acids, stimulates secretion of these hormones from intestinal K-cells and L-cells. These hormones then directly act on β -cells by binding to their specific cell-surface receptors, augmenting GSIS [140–142].

Fatty acids and insulin secretion—Free fatty acids (FFAs) also influence β -cell secretion of insulin. They potentiate insulin secretion to compensate for increased insulin need as a consequence of insulin resistance in type 2 diabetes [143–145]. FFA can also enhance GSIS. Islets deprived of fatty acids lose GSIS, which can be reversed by replacement with exogenous fatty acids [146–148]. Recently, it was discovered that β -cells have a free fatty acid receptor, free fatty acid receptor (FFAR)-1, through which FFA can influence β -cell function [149, 150]. The intracellular metabolism of FFA is the source for synthesis of lipid signal molecules such as long-chain acyl-CoA [145] and DAG [145, 151]. Long-chain acyl-CoA could acylate essential proteins in insulin granule fusion, such as synaptosomal-associated protein-25 (SNAP-25) and synaptogamin [152, 153]. DAG activates protein kinase C, which is implicated in insulin secretion [154]. It also binds to synaptic vesicle priming protein Munc-13 to promote insulin secretion [155].

Cellular signaling transduction pathways in regulation of insulin secretion

Insulin secretion is a process that involves the fusion of insulin granules with the plasma membrane and exocytosis of granule content. Insulin secretion shows a characteristic biphasic pattern that consists of a transient first phase followed by a sustained second phase. In humans, when plasma glucose is ~ 7 mM, first phase insulin secretion peaks at 1.4 nmol/min. The first phase lasts for ~ 10 min and is then followed by the second phase with the secreting rate at ~ 0.4 nmol/min [156]. However, the biphasic pattern is less prominent in mice than in rats and humans. This might be explained by the relatively higher basal plasma blood insulin levels in mice (8–9 mmol/L in mice vs. 4–5 mmol/L in rats and humans) [157, 158]. Thus, even though insulin secretion is induced by 10 mM glucose in mouse islets, the clear peak of a first phase of insulin release is missing. It is likely that biphasic insulin secretion and insulin exocytosis have the same cellular background. Although no clear boundary exists, insulin granules can be categorized into distinct functional pools [159,

160]. A small fraction of the granules (1%) are immediately available for release, named the readily releasable pool (RRP), which contribute to the rapid insulin release triggered by glucose [161]. The remaining granules (99%) belong to the reserve pool. When the RRP depletes, it is refilled from the reserve pool. Granules in the reserve pool have to undergo the preparatory reactions before becoming a RRP granule. The priming process, involving both granule modification and translocation toward the plasma membrane, is the rate limiting step for insulin exocytosis. The following observations suggest that there is a relationship between biphasic insulin secretion and pools of granules: 1). Both the first phase of insulin secretion and the exocytosis from RRP can occur even in the absence of nutrients, while both the second phase of insulin secretion and RRP replacement are strictly metabolic-product-dependent; 2), The total number of granules in RRP is positively related to the amount of insulin released in the first phase of secretion [162]; and 3). Ablation of Munc13-1 selectively suppresses second phase insulin secretion and insulin granule exocytosis, but does not affect the first phase and insulin exocytosis from the RRP [163, 164]. However, there is discrepancy in kinetics. The replacement of RRP occurs in less than 1 sec, while the first phase of insulin secretion can last for about 10 min.

Several proteins participate in insulin exocytosis. The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) plays an essential role in insulin granule membrane fusion. Four SNARE motifs form the extremely stable helical β -cell exocytotic core complex. The central part of this complex contains four highly conserved amino acids contributed by the four SNARE motifs: three glutamine (Q), and one arginine (R) residue [165]. In β -cells, the fusion of the insulin granules with the plasma membrane involves the assembly of a complex consisting of VAMP-2 (R-SNARE) on the granule membrane, syntaxin-1a (Qc-SNARE) on the plasma membrane, and the membrane-associated protein SNAP-25 (Qa-Qb SNARE). The assembly of this complex can be regulated by other accessory factors to achieve elegant regulation of insulin granule fusion. Tomosyn-1 is such a regulatory factor that can replace VAMP2 in the process of assembly [166]. It is required for granule fusion and/or priming of the granules, but its absence does not influence insulin granule transport and docking [167, 168].

The priming and fusion of insulin granules, which results in insulin exocytosis, is triggered by elevation of intracellular $[Ca^{2+}]$. Insulin exocytosis can proceed at the rate of 500 granules per second when intracellular $[Ca^{2+}]$ is increased to 17 mmol/L, but it only proceeds at the rate of 3–4 granules per second when $[Ca^{2+}]$ is at 0.17 mmol/L. Exocytosis occurring at low $[Ca^{2+}]$ is due to a small portion of granules capable of releasing insulin, which are referred to as the high Ca^{2+} -sensitive pool (HCSP). The exocytosis occurring at different rates is controlled by two Ca^{2+} sensing mechanisms: the low affinity Ca^{2+} sensor and the high affinity Ca^{2+} sensor. Exocytosis occurring at high $[Ca^{2+}]$ are controlled by the low affinity Ca^{2+} sensor. Synaptotagmin IX was reported to be a high-affinity Ca^{2+} sensor in β -cells [169] and it remains to be determined if synaptotagmin IX also functions as a low-affinity Ca^{2+} sensor. Another putative Ca^{2+} sensor is piccolo, which facilitates rapid Ca^{2+} -induced exocytosis by interacting with essential proteins, including cAMP-regulated guanine nucleotide exchange factor/exchange proteins activated directly by cyclic AMP (cAMPGEFII/Epac2), sulfonylurea receptor1 (SUR1), and the L-type Ca^{2+} channels [158, 170, 171]. Thus, piccolo might act as a low-affinity Ca^{2+} sensor.

Intracellular $[Ca^{2+}]$ is determined by the open and closure of plasma membrane Ca^{2+} channels. Three subfamilies of voltage-gated Ca^{2+} channels exist: (1). L-type high voltage-activated (HVA) Ca^{2+} channels. They are sensitive to dihydropyridines (DHP) and include (1) $CaV1.1$, 1.2, 1.3, and 1.4 channels [172–174]; (2) the non-L-type HVA channels $CaV2.1$ (P/Q-type), 2.2 (N-type) and 2.3 (R-type) [172, 173, 175]; and (3) the low voltage-activated (LVA) T-type Ca^{2+} channel ($CaV3.1$, 3.2, and 3.3). LVA differs from HVA Ca^{2+} channels

electrophysiologically. The LVA open transiently upon modest depolarization [176, 177]. They are pacemakers in most cell types [178]. A mixture of voltage-gated Ca^{2+} channels were reported to be present in β -cells [179–181]. The existence of L-type Ca^{2+} channels was first confirmed about 25 years ago by radioisotopic and electrophysiological measurements [182]. Later the expression of $\text{CaV}1.2$ of the L-type Ca^{2+} channels, and P/Q, N and R-type Ca^{2+} channels were confirmed by single-cell PCR [181]. The first phase of insulin secretion couples to the activation of L-type $\text{CaV}1.2$ channels, whereas the second phase secretion depends on R-type ($\text{CaV}2.3$ channels), which mediates a moderate global increase in intracellular $[\text{Ca}^{2+}]$. The R-type Ca^{2+} channel-mediated Ca^{2+} influx is insufficient to cause insulin exocytosis, but accelerates granule mobilization, and increases the size of RRP [183].

Glucose induces insulin secretion by both triggering (i.e. involving closure of the K_{ATP} channels) and amplifying (i.e. post K_{ATP} channel closure) effects. Although the increase in intracellular $[\text{Ca}^{2+}]$ is the primary signal that triggers insulin exocytosis by glucose, there are other cell signals activated by glucose that also play roles in this process, such as cAMP, cGMP, inositol 1,4,5-trisphosphate (IP3), and DAG [183]. Among those signaling molecules, cAMP may be the most important one for potentiating insulin secretion [154, 184–187]. Around 50 years ago, it was found that an oral glucose load elicits greater insulin secretion than an intravenous glucose load even though similar circulating glucose levels were achieved by these two methods [187]. The potentiated insulin secretion by orally ingested glucose was due to the action of GIP and GLP-1, incretin hormones secreted by enteroendocrine K cells and L cells, respectively, upon glucose ingestion [188, 189]. Incretin hormones augment GSIS by stimulating the cAMP signaling pathway. Cyclic AMP's action was generally thought to be mediated exclusively by the activation of protein kinase A (PKA), which phosphorylates proteins involved in insulin exocytosis [190]. However, the insulinotropic effect of cAMP can only be blocked partially by inhibition of PKA activity, suggesting that an alternative mechanism exists that mediates, in part, the cAMP effect on insulin exocytosis. Recently it was discovered that cAMP stimulates exocytosis of insulin granules from RRP, an effect that was unaffected by PKA inhibition, suggesting a PKA-independent mechanism [191]. A cAMP-binding protein called CAMPS (cAMP sensor) was identified by yeast two-hybrid screening of the insulinoma cell line MIN6, during the search for intracellular molecules that interact with the sulfonylurea receptor SUR1 [192]. CAMPS was later identified using a BLAST-search approach as a mouse homolog of rat cAMP-GEFII/Epac2, which is an isoform of cAMP-GEFI/Epac1 [193, 194]. Studies using a yeast two-hybrid system later confirmed the interaction between cAMP-GEFII/Epac2 and SUR1 [192, 195], revealing a novel cAMP-GEFII/Epac-dependent pathway activated by cAMP.

The cAMP-binding protein, cAMP-GEF/Epac participates in potentiating insulin secretion in a PKA-independent manner. cAMPGEFII/Epac2 is abundant in the brain and neuroendocrine and endocrine tissues including pituitary, adrenal, and pancreatic islets, while cAMP-GEFI/Epac1 is expressed at high levels in adult tissues including thyroid, kidney, ovary, skeletal muscle, and heart, and at low levels in the brain [192–194]. In addition to SUR1, which is the regulatory subunit of the K_{ATP} channel, cAMPGEFII/Epac2 also binds to Rab3-interacting molecule 2 (Rim2) [192] and Piccolo [196]. Rim2 is the target of the small G-protein Rab3, which is involved in exocytosis [197]. Piccolo defines and organizes the site of neurotransmitter release in neurons [198]. Piccolo also forms both homodimers and heterodimers with Rim2 in a Ca^{2+} -dependent manner [196]. cAMP-GEF/Epac, which has a higher dissociation constant for cAMP ($1.2\text{--}4\mu\text{mol/L}$, PKA: $5\text{--}25\mu\text{mol/L}$), is a cAMP sensor when PKA activity is fully saturated [199–201]. The cAMP-GEF/Epac might be localized in cAMP compartments distinct from PKA, since a much higher concentration of cAMP is required for activating the cAMP-GEF/Epac-mediated signaling than that for stimulating PKA activity. In the absence of cAMP, Ras exchange motif (REM)

binds to GEF/Epac to stabilize GEF/Epac and inhibit its activity. Cyclic AMP activates GEF/Epac by binding to its regulatory region. Activated GEF/Epac then activates Ras-like small GTP-binding proteins, Rap1 and Rap2 [193, 194, 199, 202]. Cyclic AMP-GEFII/Epac2 may be involved in both the first and second phase of insulin release, since treatment with antisense oligodeoxynucleotides (ODNs) against GEFII/Epac2 in pancreatic islets reduced both first and second phases of cAMP- potentiated insulin secretion [203]. Interaction between SUR1 and cAMP-GEFII/Epac2 is an essential step in this PKA-independent cAMP potentiated insulin secretion. This PKA-independent effect on cAMP-regulated insulin secretion is impaired in SUR1 knockout islets [204, 205] and early PKA-independent exocytosis is absent in SUR1 knockout β -cells [170]. Being recruited to the plasma membrane by SUR1, cAMP-GEFII/Epac2 mediates the cAMP-dependent activation of Rap GTPase activity. Rap then stimulates phospholipase C (PLC)- ϵ , which catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2). The hydrolysis of PIP2 causes inhibition of K_{ATP} channels [206]. Therefore, the interaction between cAMP-GEFII/Epac2 and SUR1 is independent of intracellular ATP concentration [207]. After activated by cAMP, cAMP-GEFII/Epac2 dissociates from the SUR1-cAMP-GEFII/Epac2 complex, which then releases its inhibition on K_{ATP} channels. There is another hypothesis that after activated by cAMP, cAMP-GEFII/Epac2 will dissociate from granule SUR (gSUR, a putative SUR on insulin granules) to open a chloride channel (ClC-3), which is coupled with gSUR. Opening of the ClC-3 channel allows Cl^- influx to promote granular acidification, which allows insulin granule priming and refilling of the RRP [208]. Accumulating evidence also suggests that activation of cAMP-GEFII/Epac2 mobilizes Ca^{2+} from intracellular Ca^{2+} storage, thereby increasing insulin secretion [209–212]. Three possible mechanisms might mediate this Ca^{2+} mobilization: 1). cAMP-GEFII/Epac2 interacts with IP3 receptors and ryanodine receptors to increase intracellular Ca^{2+} channel sensitivity to Ca^{2+} or Ca^{2+} mobilizing signals; 2). cAMP-GEFII/Epac2 might act through Rap and extracellular signal regulated kinase to sensitize intracellular Ca^{2+} release; and 3). cAMPGEFII/Epac2 acts through Rap to stimulate PLC- ϵ , thereby hydrolyzing PIP2 to release IP3, which signals release of Ca^{2+} from the ER [213].

Hormone regulation of insulin secretion

Estrogen— β -cells are not considered classic estrogen targets; however, estrogen receptors are present in islets [214] and the effects of 17β -estradiol on β -cells are well documented [215]. The main physiological consequence of 17β -estradiol action on β -cells is the enhancement of insulin secretion [216]. In humans, 17β -estradiol can increase insulin secretion in postmenopausal women [217, 218]. This insulinotropic effect is mediated by potentiating glucose-stimulated insulin secretion (GSIS) [219]. The effects of estradiol are initiated by its binding to estrogen receptors. Two types of estrogen receptor (ER) are present in β -cells: 1). the nuclear ERs (ER α and ER β) and 2). the membrane ER (ER γ) [220]. It is reported that at physiological concentrations, 17β -estradiol can significantly decrease K_{ATP} channel activity in a reversible manner [216], which causes membrane depolarization and subsequent opening of voltage-gated Ca^{2+} channels, thereby potentiating glucose-induced intracellular $[Ca^{2+}]$ oscillations. The modulation of K_{ATP} channel activity by estradiol may be mediated by activation of the cGMP-dependent protein kinase (PKG) pathways [221]. The activated PKG can directly phosphorylate transcriptional factor CREB. After phosphorylation, CREB can bind to CRE, which in turn modulates transcription of genes containing cAMP/ Ca^{2+} response elements to potentiate glucose-induced intracellular $[Ca^{2+}]$ oscillations that influence insulin secretion [222–225].

Melatonin—Melatonin is a hormone secreted by the pineal gland, which helps adjust the timing or reinforces oscillations of the biological clock [226]. The direct effect of melatonin on β -cells was confirmed by the discovery of melatonin receptors on both clonal β -cells

[227, 228] and human islets [229]. Effects on insulin secretion are controversial. There are studies showing that melatonin exerts an inhibitory [230, 231], neutral [232], or stimulatory effect on insulin secretion [233]. However the inhibitory effect is consistent in replicated experiments with clonal β -cells [227, 229, 233, 234]. Melatonin attenuated glucose- and KCl-stimulated insulin secretion in rat islets [235]. The inhibitory effect of melatonin on insulin release was later confirmed in rat islets [236]. Consistently, chronic melatonin administration ameliorates hyperinsulinemia in vivo [237].

It was reported that melatonin receptor is coupled to G_i , which inhibits G protein [238]. G protein activation will further activate adenylate cyclase to catalyze cAMP production. Indeed, melatonin blocks the enhanced insulin secretion by cAMP agonists forskolin or GLP-1 [227, 228]. In contrast, cAMP levels in human islets are not influenced by melatonin, whereas the formation of cAMP in MIN-6 cells is impaired in the presence of melatonin [229]. Melatonin also decreased cGMP levels to inhibit insulin secretion. This effect is mediated by activation of melatonin receptor (MTNR) 1B [239]. However, when G_i coupling is blocked by pertussis toxin, MTNR1A also mediated a stimulatory effect on insulin secretion by coupling to $G_q/11$. The activation of $G_q/11$ provokes the release of IP3 by activating PLC- ϵ to potentiate insulin secretion [233, 240, 241].

GLP-1—GLP-1, an incretin hormone, is secreted from small intestinal L-cells together with GIP in response to nutrient load [242, 243]. Incretin is responsible for augmentation of insulin secretion to meet the increased demand for insulin after a meal. Experiments have shown nutrient load from the oral route stimulates more insulin secretion than intravenous nutrient load [244]. The analogs of both GLP-1 and GIP have been explored as a potential therapy for T2D for many years, and the long-lasting GLP-1 analog exenatide was introduced to clinics in 2005 as a prescription drug for T2D treatment [245]. Upon activation of the GLP-1 receptor (GLP-1R), adenylyl cyclase is activated, leading to the generation of cAMP [246]. The elevated cAMP then potentiates GSIS. This insulinotropic effect is dependent on glucose. When the extracellular glucose concentration is in the normal fasting range (lower than 4 mmol/L), GLP-1 is inactive in stimulating insulin secretion [245]. Such glucose-dependent action of GLP-1 is very important in preventing hypoglycemia.

Leptin—Leptin is secreted by adipocytes and is known to influence insulin action in fat and liver cells [247, 248]. It is generally accepted that leptin exerts an inhibitory effect on insulin secretion. Leptin deficiencies are associated with hyperinsulinemia in both mice and humans [247, 249]. A large body of literature shows that leptin plays an inhibitory role in insulin secretion in clonal β -cells [250–252], cultured rodent islets [251–259], human islets [251, 260, 261], perfused rodent pancreas [250, 262], as well as in mice [251]. It is hypothesized that leptin's inhibitory effect is through antagonizing the action of elevated intracellular cAMP [263], since it was reported that leptin inhibits insulin secretion induced by 3-isobutyl-1-methylxanthine (IBMX), which elevates cAMP content by inhibiting phosphodiesterases (PDEs) [258], the enzymes catalyzing the hydrolysis of cAMP. Leptin also potently inhibits glucagon-like peptide-1 (GLP-1)-induced insulin secretion, which augments GSIS through activation of the cAMP signaling pathways [252, 262]. Leptin was shown to inhibit insulin secretion by activating PDE 3B, a subtype of PDE [252].

Growth hormone—Growth hormone (GH) has targets in variety of cells but one of its best-known actions is to stimulate production of insulin-like growth factor-I (IGF-I) and its binding proteins [264]. Recombinant human IGF-I was shown to decrease serum levels of insulin and C-peptide in normal human subjects [265]. Ex-vivo studies using isolated rat islets confirmed that IGF-1 directly suppresses insulin secretion [266]. This inhibitory effect is possibly mediated through activation of PDE3B [267], which is responsible for breaking down cAMP in β -cells, as stated above.

Pancreatic β -cell apoptosis and regeneration

Both T1D and T2D are characterized by progressive β -cell destruction, of which apoptosis is the main form. Although β -cell loss is caused by excessive nutrients in T2D and an autoimmune reaction in T1D, there is a convergence in cellular signaling pathways between the two types of diabetes [268], which is schematically illustrated in Fig (2).

T1D is a T-cell-mediated autoimmune disease resulting from selective destruction of pancreatic β -cells. The incidence of T1D is estimated to increase from 4.4 million in 2000 to about 5.4 million in 2010 [269]. However the pathogenic mechanisms and T-cell mediated autoimmune process that destroy pancreatic β -cells in T1D are complex and are not fully defined, the subject of many excellent reviews [269–272]. It is clear from past studies that infiltration of inflammatory cells, such as T helper type 1 (Th1) cells and macrophages into the islets in response to islet associated antigens and subsequent insulinitis are hallmarks of the pathogenesis of T1D. Activated T cells and macrophages release several proinflammatory cytokines, such as interleukin-1 β (IL-1 β), interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α), which are believed to be important mediators leading to β -cell destruction in T1D [273–278]. These cytokines act on β -cells through their specific receptors to induce several signal transduction pathways that lead to alternations in gene and protein expressions [269]. Research evidence suggests that activation of NF- κ B may be a common and crucial step for various cytokine-stimulated β -cell dysfunction. Activation of NF- κ B will lead to induction of its downstream gene inducible nitric oxide (NO) synthase (iNOS) and subsequent NO production [279]. Cumulative evidence shows that IL-1 β , IFN- γ and TNF- α induce the overexpression of iNOS in β -cells, leading to the overproduction of NO that causes the cytotoxicity to β -cells [278], suggesting an important role for NO in the pathogenesis of diabetes. Indeed, transgenic mice overexpressing iNOS in β -cells developed insulin-dependent diabetes [280]. Conversely, inhibition or knockout of iNOS in the islets protects β -cells in vitro and in vivo from the cytotoxic effects of cytokines [281–283]. While additional mechanisms may also be involved in the pathogenesis of T1D, these data clearly indicate that iNOS derived NO is at least partially responsible for cytokine-mediated destruction of β -cells, which is central to the development of T1D [284–287]. β -cell proliferation is increased during the pathogenesis of diabetes in humans and non-obese diabetic (NOD) mice, an animal model for human T1D [288, 289], which does not adequately compensate for autoimmune-mediated destruction of β -cells [290, 291]. As such, identifying agents that can simultaneously induce β -cell proliferation and survival can provide novel treatment for T1D.

β -cell apoptosis in the course of insulinitis in T1D is caused by direct contact with islet infiltrated T cells and macrophages and exposure to soluble mediators secreted by those infiltrated immune cells such as oxygen free radicals, NO, and cytokines including IL-1 β , IFN- γ and TNF- α [292]. IL-1 β and IFN- γ are considered to be two major soluble factors which mediate β -cell damage. In vitro cell culture showed that exposure of β -cells to IL-1 β or IL-1 β +IFN- γ causes β -cell destruction similar to that which was observed in pre-diabetic patients [293]. In response to the stimulation of IL-1 β and IFN- γ around 700 genes are up- or down-regulated in β -cells [294].

In β -cells, IL-1 β can activate transcription factor nuclear factor (NF)- κ B [292]. Although basal NF- κ B activity is required for maintaining β -cell normal insulin secretory function [295], excessive activated NF- κ B will result in up-regulated expression of inducible nitric oxide synthase (iNOS) [296]. iNOS can produce massive amounts of NO which will result in decreased expression levels of transcription factors responsible for β -cell differentiation and function (e.g., PDX-1 and Isl-1) [297, 298]. This cytokine mediated NF- κ B also up-regulates chemokines such as monocyte chemoattractant protein-1 (MCP-1) [299, 300] and down-regulates the Ca²⁺ pump sarcoendoplasmic reticulum Ca²⁺ ATPase type 2b

(SERCA-2b) [298, 301] Decreased SERCA-2b expression leads to ER calcium depletion and severe ER stress which results in β -cell apoptosis [298, 302–304].

Exposure to IL-1 β also activates the c-Jun NH2-terminal kinase (JNK), a member of the mitogen-activated protein kinases (MAPKs) [305, 306]. JNK plays an important role in the intracellular events during β -cell loss [307]. Cell-permeable peptide inhibitors of JNK prevent cytokine-induced β -cell apoptosis [308]. IFN- γ is reported to synergize with IL-1 β to trigger β -cell apoptosis [292]. IFN- γ binds to cell surface receptors and activates JAK1 and JAK2, which phosphorylate the transcription factor STAT-1. Upon activation STAT-1 forms a dimer and translocates into the nucleus to activate γ -activated site of diverse genes including up-regulating iNOS expression [292, 296].

Cytokines and hyperglycemia share some common mechanisms to alter β -cell gene expression. C-Myc, A20, and heme-oxygenase are induced under both conditions. Hyperglycemia was reported to increase the production of IL-1 β from β -cell [309]. However, the patterns of hyperglycemia-induced genes in β -cells are not exactly the same as that induced by cytokines [294, 298, 310] suggesting that there is a divergence in mechanisms of β -cell apoptosis between these two conditions. NF- κ B dependent genes which are strictly activated by IL-1 β remained unchanged after high glucose exposure, while lactate dehydrogenase A, the mitochondrial uncoupling protein (UCP)-2, and the transcription factor cAMP-responsive element modulator (CREM) can be induced in hyperglycemia [310, 311]. β -cell glucotoxicity at least in part results from an increase in β -cell oxidative stress, and subsequent JNK activation is NF- κ B independent [312, 313]. The main source of oxidative stress probably comes from the mitochondrial electron transport chain [314, 315]. In addition, ER stress and sustained elevation of cytosolic calcium concentrations could also be possible explanations of β -cell viability loss [316].

Besides glucotoxicity, high plasma concentration of free fatty acid (FFA) is another risk factor for β -cell destruction. The effect of dyslipidemia depends on the lipid profile. Saturated fatty acids such as palmitate are highly toxic at long-term exposure, whereas monounsaturated fatty acids such as oleate protect β -cells from palmitate and high glucose-induced β -cell apoptosis [317, 318]. FFA's toxicity on β -cells is suggested to be iNOS/NO independent, because of the absence of iNOS expression or NO production in FFA-mediated β -cell apoptosis [319, 320]. It is also reported that FFA's toxic effect is oxidative stress independent [320]. Moreover, oleate or palmitate did not activate NF- κ B in β -cells [319]. FFA-induced β -cell toxicity might occur at the ER level where FFAs are esterified. Both oleate and palmitate can induce ER stress markers including alternative splicing of XBP-1, activation of ATF-6, and induction of the ER chaperone BiP [319]. Besides, FFA might also impair ER calcium handling [321]. Therefore, in the condition of increased insulin demand such as high glucose, ER stress induced by FFA might be amplified.

In mammals, β -cells have a slow turnover rate. In healthy adult individuals, there is an extremely low ratio of replicating β -cells to apoptotic ones [322]. However, β -cell proliferation can be increased in obese or/and insulin resistant individuals [323–325], during the progression of autoimmunity in type 1 diabetes [326], during pregnancy [327], and in response to mechanical or chemical damage [327], demonstrating the plasticity of the tissue. The population of β -cells can be increased by several mechanisms: replication of the existing β -cells, neogenesis from pancreatic precursor 'stem cells' [328], and as recently discovered, trans-differentiation from fully-differentiated α -cells into β -cells [327]. Trans-differentiation was initiated in the pancreas of mice during recovery after chemical-induced destruction of β -cells, with 30–80% of regenerated β -cells derived from α -cells [327]. Besides the increase in cell number, an increase in cell size also contributes to increased β -cell mass to meet a greater insulin requirement. The mechanism by which β -cells expands is

hypothesized to involve up-regulation of protein synthesis, but the exact molecular mechanism is unclear [329].

β -cell proliferation and differentiation can be influenced by certain nutrients and various growth factors. Nutrients that simulate insulin secretion and synthesis can also increase β -cell proliferation. Glucose is the most physiological relevant β -cell growth-promoting nutrient [330, 331]. Some of the growth factors that can stimulate β -cell growth, such as IGF-1 and GH, are glucose-dependent [331, 332]. However, glucose-mediated β -cell growth is largely an acute effect. Chronic exposure to high glucose will evoke β -cell apoptosis [333].

GH stimulates β -cell growth by binding to growth hormone receptor (GHR) present on β -cell which leads to JAK-mediated tyrosine phosphorylation and activation of STAT5a and 5b [334, 335]. Activated STAT5a and 5b then up-regulate cyclin D2 expression, which is an essential regulator of β -cell proliferation [336, 337]. In some types of cells, GH's effect is mediated by increasing local IGF-1 production. But β -cell IGF-1 and GH signal transduction pathways are independent [332, 338]. IGF-1 mediated β -cell mitogenesis requires the induction of phosphatidylinositol 3-kinase (PI3K) activity, which is located downstream of insulin receptor substrate (IRS)-2 [331]. IGF-1 will activate protein kinase-B (PKB; also known as Akt) which is important for β -cell survival [339, 340]. PKB in turn can phosphorylate glycogen synthase kinase (GSK)-3 [341, 342] leading to inhibition of GSK-3 [343]. Although the consequences of GSK3 inactivation are currently unclear, GSK-3 is able to control general protein synthesis and cell differentiation which contribute to β -cell hypertrophy and neogenesis [343].

THE PATHOGENESIS OF T1D

A decrease in both mass and insulin secretory function of β -cells is the common characteristic shared in both type 1 and type 2 diabetic patients. Autoimmunity plays a critical role in the development of T1D. The classical T1D is characterized by the presence of antibody (humoral) and T-cell (cellular) responses to self-islet proteins (antigens) [344–348]. Histological analysis of the pancreas from patients with T1D shows the presence of immunological activity [349]. Drugs that suppress the immune response such as cyclosporine and azathioprine can slow the progression of β -cell destruction pointing to the critical role of immune activity in development of type 1 diabetes [350, 351]. Development of T1D may be influenced by dietary factors including early infant feeding status [352], Vitamin D and omega 3 polyunsaturated fatty acid intake [353], and duration of exposure to gluten [354]. People with genetic predispositions have a higher risk to develop overt T1D. Human leukocyte antigen (HLA) encodes cell surface proteins that interact with immune cells and are an important gene family that contributes up to 40% of T1D risk. The HLA Class II region is considered to be the most influential. In Caucasians, HLA types DR3-DQA 0501-DQB1 0201 and DR4-DQA1 0301-DQB1 0302 are strongly associated with risk, while DQB1 0602 is associated with protection [355].

Innate immune response signaling is involved in the initiation of the autoimmune process. However, the molecular pathways of innate immunity linked to T1D development are yet to be elucidated [356]. Adaptive immunity is known to play a critical role in β -cell destruction in T1D development. Humoral and cellular immunity are the two major facets of adaptive immunity. In T1D, the appearance of multiple autoantibodies is believed to reflect progressive β -cell autoimmunity [357, 358]. Although the autoantibodies can be markers for T1D, whether they contribute to the pathogenesis is not confirmed [358]. β -cell destruction is partly mediated by the cellular immune response. A schematic illustration of immunological aspect of T1D is shown in Fig. (2).

T lymphocytes are reported to be the primary mediator in T1D progression [359], though macrophages and dendritic cells infiltrate islets before T lymphocytes [360]. The indispensable role of T lymphocytes is supported by their presence in insulinitis, and detection of circulating autoreactive T lymphocytes in clinical overt T1D patients, and the observation that immunosuppressive drugs specifically against T lymphocytes delay disease progression [361]. Although T1D is T lymphocyte-dependent, paradoxically both NOD mice and human T1D patients are lymphopenic [362–365]. The decreased number of T lymphocytes drives T lymphocyte homeostatic expansion [365]. This homeostatic expansion results in increased effector/memory T lymphocytes instead of naive T lymphocytes [366]. These effector/memory T lymphocytes can generate new effector cells more efficiently, which precipitate autoimmune disease [367]. Two subsets of T lymphocytes, CD4⁺ and CD8⁺, are both involved in T1D development. The precise role of CD4⁺ and CD8⁺ T lymphocytes in β -cell destruction are controversial. It is generally accepted that CD4⁺ T lymphocytes contribute to provide proper homing for CD8⁺ effector cells as well as being effector cells themselves [368].

Naive CD4⁺ T lymphocytes reside as T helper (Th) 0 cells in secondary lymphoid organs before they encounter antigens. After encountering antigens they differentiate into functional subsets namely Th1 secreting IL-2, INF- γ , and TNF- α [369, 370] and Th2 secreting IL-4, IL-5, IL-10 and IL-13 [371–374]. Studies about the correlation of diabetes and T helper cell phenotypes lead to the idea that Th1 and their cytokines promote diabetes [374, 375]. Th1 cells can either cause β -cell destruction directly [376, 377] or by secreting Th1 cytokine (INF- γ) to recruit and activate macrophages and CD8⁺ T lymphocytes that exert toxic functions [378]. In contrast, Th2, through secretion of the cytokine IL-4, is generally considered protective [375, 379]. Although there is evidence that Th2 plays a role in causing β -cell damage, it is through IL-10 instead of IL-4 [380–382].

Th1/Th2 differentiation is influenced by the antigen concentration, ligation of co-stimulatory molecules, and cytokine circumstance; but eventually transcription factors T-bet and GATA-3 control T helper cell differentiation [383–385]. Cells dominated by T-bet will differentiate into Th1 cells, while Th2 differentiation is directed by GATA-3 expression [385]. The activation of Th1 and Th2 cells can be suppressed by a specialized subpopulation of CD4⁺ T cells named regulatory T (Treg) cells. Treg cells, which are protective, contribute to immune suppression by suppressing activity of both CD4⁺ T lymphocytes and CD8⁺ cytotoxic T lymphocytes [386–388]. Treg cells comprise 5–10% of the peripheral CD4⁺ T lymphocyte population in mice and humans [389]. Naturally occurring Treg cells (nTreg) are generated in thymus and express surface markers CD4 and CD25, and an intracellular marker, transcription factor forkhead box P3 (Foxp3) [390]. The other Treg subpopulation named induced Treg (iTreg) is generated in response to antigen. They are not CD25⁺ by default. But they share features with nTreg in terms of Foxp3 expression and bystander (non-antigen-specific) immune suppression [390].

Similar to CD4⁺ T lymphocytes, mature CD8⁺ T lymphocytes reside as naive cells in secondary lymphoid organs. After encountering self-antigen [391], activated CD8⁺ T cell differentiate into effector cells. Activated CD8⁺ T cells destroy β -cells either through a perforin-dependent pathway or alternatively by the Fas/FasL pathway [373, 392]. The pore-forming protein perforin and the granzyme B are key constituents of cytolytic granules. After conjugate formation, perforin and granzyme are released toward the target cell membrane where they synergize to cause apoptotic cell death [393]. INF- γ is reported to be crucial in activating the Fas/FasL pathway in islets. [392]. Therefore, INF- γ and granzyme B levels are markers of CD8⁺ T cell-induced β -cell damage. Similar to CD4⁺ cells, the activity of CD8⁺ cells can be tuned at the genetic level. T-bet, known to regulate Th1 cell differentiation, also controls the differentiation of the CD8⁺ cytotoxic effector cell [394].

Recently, it was reported that the activity of Th17 cell, which is a newly discovered subpopulation of CD4⁺ T lymphocytes secreting IL-17, is associated with autoimmune conditions in a variety of autoimmune diseases including rheumatoid arthritis [395], inflammatory bowel disease [396], and multiple sclerosis [397]. Studies have shown that Th17 cells may up-regulate IFN- γ and also extinguish IL-17 in response to IL-12 or IL-23 in the absence of TGF- β in vitro and depreciated to a Th17/1 (IL-17+IFN- γ +) or Th1 phenotype [398, 399]. Although the relative contribution of Th17 cells in T1D is not well defined, results from studies showed that high level of IL-17 transcripts were found within insulinitic lesions in NOD mice [400]. Increasing levels of serum IL-17 is associated with accelerated disease progression in a T cell receptor transgenic NOD model [400]. More recently, the protective effect of therapeutic intervention with an antigen-specific agent is associated with a decrease in the Th17 population [401]. However, the specific contribution of this subpopulation of CD4⁺ T lymphocytes to the natural progression of T1D remains to be fully characterized.

THE PATHOGENESIS OF T2D

T2D is a result of chronic insulin resistance and loss of β -cell mass and function [284]. Both in experimental animals and people, obesity is a leading pathogenic factor for developing insulin resistance, which is always associated with impairment in energy metabolism, causing increased intracellular fat content in skeletal muscle, liver and fat, as well as pancreatic islets. Chronic insulin resistance will progress to T2D when β -cells are unable to secrete adequate amounts of insulin to compensate for decreased insulin sensitivity, which is largely due to insulin secretory dysfunction and significant loss of functional β -cells [284–287, 402, 403]. Indeed, those individuals with T2D always manifest increased β -cell apoptosis and reduced β -cell mass [286, 287, 404]. Progression to full-blown T2D involves insulin resistance leading to β -cell dysfunction [405–407]. Insulin resistance is observed in a variety of patient conditions including gestational diabetes, obesity, impaired glucose tolerance (IGT) and polycystic ovarian syndrome [408, 409]. Although obesity is associated with T2D, most obese people don't develop the disease and increased insulin secretion due to enhanced function of pre-existing β -cells or expansion of β -cell mass compensates and restores blood glucose levels [410]. Enhanced functionality involves increased nutrient signals stimulating increased growth factor signaling in β -cells [406]. In particular, increased nutrient load in the gut can enhance GLP-1 production leading to the anti-apoptotic and growth-promoting effects on β -cells [411, 412]. In “susceptible” individuals compensation becomes insufficient and cell dysfunction ensues. Generally, diagnosis of T2D is associated with an approximate 50 % reduction in islet function and this is thought to manifest itself at least 10–12 yr prior to diagnosis, a condition exacerbated by elevated fasting blood glucose [413]. Obese non-diabetic humans show increased relative β -cell volume in islets while obese and non-obese patients with impaired fasting glucose and T2D show at least a 40 % reduction in β -cell volume compared with respective non-diabetic patients [404]. Apoptosis of β -cells was substantially increased in all diabetic patients and was implicated as the primary mechanism underlying the decrease in β -cell mass in T2D individuals although β -cell mass is controlled by several factors including cell size, rate of cell renewal from proliferation of pre-existing cells or neogenesis (differentiation from other precursor cells) and rate of apoptosis. As the number of β -cells per islet declines in T2D patients, islet space becomes dominated by amyloid plaque deposits although the role of islet amyloid deposits in β -cell dysfunction is unclear [414]. The factors leading to a change in β -cell function (decreased insulin expression and secretion) and mass are central to the pathology of T2D.

The prevailing theories for causes of β -cell failure during the progression to T2D involve chronic exposure of the β -cell to glucose and fatty acids, also known as “glucotoxicity” and “lipotoxicity”, respectively [415–420]. It is known that transient exposure of islets to free

fatty acids (FAAs)(e.g., hours) can augment GSIS whereas long-term exposure (e.g., days) decreases insulin secretion. In general, it is accepted that hyperglycemia precedes conditions for lipotoxicity while glucotoxicity can occur independently of lipotoxicity [310, 421]. Taking this idea a step further, the combination of these factors is known as “glucolipotoxicity”. We define “glucolipotoxicity” as chronic exposure of the islets to greater-than-physiological concentrations of glucose and fatty acids, leading to β -cell damage [312]. The following sections will dissect the meaning of these terms and some of the cellular and molecular mechanisms by which these phenomena alter β -cell function, with a particular emphasis on insulin synthesis and secretion. A schematic illustration of these processes is also included in Fig. (2). Bear in mind that differences in model system (in vitro vs. in vivo, primary vs. clonal cell lines (MIN6, INS1, HIT-T15, BetaTC-6), rodent vs. human, genetic vs. nongenetic), age of animal, concentrations of substrates (fatty acids, glucose), length of incubation or exposure (minutes vs. hours vs. days), etc. can all influence the outcome, making it very difficult to draw a definitive picture of β -cell pathology and diabetes mellitus. Studies described in this section involve primary cultured rat, human and mouse islet cells, as well as clonal β -cell clones and tissues harvested from humans and rodents.

Glutotoxicity -glycation and reactive oxygen species (ROS) production in the β -cells

While exact mechanisms are debatable, the general consensus is that long-term elevated levels of glucose have deleterious consequences for β -cells, which rely on signals of energy status to control metabolism [420]. Abundant expression of the low-affinity high-capacity GLUT2 in β -cells coupled to the role of glucose in stimulating insulin synthesis and secretion leads to excessive glucose concentrations in β -cells and effects on β -cell metabolic pathways. Glucotoxicity also down-regulates GLUT4 levels in insulin-responsive cells [405]. Chronic exposure to glucose leads to increases in cytosolic calcium that induce β -cell destruction [422]. It also leads to increased production of IL-1 β , subsequent NF- κ B activation, increases in FAS, DNA fragmentation and damaged β -cell function [423, 424].

Hyperglycemia leads to glycation reactions and production of ROS [425]. Glycation occurs non-enzymatically and can alter the function of a variety of molecules, and advanced glycation end products (AGEs) are implicated in cellular damage [426]. The antioxidant and glycation-inhibitor aminoguanidine, which prevents formation of AGEs and ROS, is able to partly ameliorate the effects of those damaging compounds on β -cell function [426], similar to the beneficial effect of the hydrogen peroxide-scavenging *N*-acetyl-L-cysteine on insulin expression and secretion in *db/db* mice or Zucker rat β -cells subjected to oxidative stress [427, 428]. The importance of ROS in β -cell pathology is supported by the observation that 8-hydroxy-2'-deoxyguanosine (8-OHdG) an oxidative stress marker, is elevated in β -cells from diabetic Goto-Kakizaki (GK) rats [429] and that the insulin [430] and glucokinase [425] promoters are sensitive to glycation and the presence of ROS (superoxide, hydrogen peroxide, nitric oxide, hydroxyl radicals). Furthermore, there are elevated levels of oxidative stress markers in the blood and urine of T2D patients, and reduced glutathione (GSH) in blood cells [312]. Fructose, D-ribose and 2-deoxy-D-ribose have a greater reducing capacity than glucose [431].

The process of aging, which involves long-term exposure to ROS, increases in body weight, a sedentary lifestyle, and reduced functionality of β -cells, partly accounts for prevalence of this disease in middle-aged to older adults [405]. Counter intuitively perhaps, the β -cell expresses relatively low levels of antioxidant enzymes, including CuZn superoxide dismutase (SOD), Mn-SOD, catalase, and glutathione peroxidase (GPx) [312, 432–435]. The Mn-SOD functions in the mitochondria, Cu/Zn-SOD in the cytosol, and both catalyze generation of hydrogen peroxide from the reaction of superoxide and hydrogen [312]. The

GPx species reduce hydrogen peroxide to water with GSH, and also lipid peroxides to alcohols. Oxidized GSH (GSSG) can be converted back into GSH by GSH reductase using NADPH as a cofactor. Islet ROS levels were correlated with glucose concentration [435]. Blocking the existing GPx activity with buthioinine sulfoximine, an indirect inhibitor of GSH synthesis, hampered the beneficial effect of N-acetylcysteine, an antioxidant, on ribose-induced decreases in insulin production [435]. Isolated islets transfected with GPx exhibited a six-fold increase in enzyme activity, which negated the detrimental effects of ribose.

Hydroxyl radicals are particularly dangerous in β -cells because of their ability to cross the nuclear membrane and exert a mutagenic effect [312]. The β -cells are particularly vulnerable to oxidative stress [312]. Oxidative phosphorylation generates ROS [436], as well as other pathways for glucose when glycolytic enzyme activity becomes saturated: glycosylation (Schiff reactions), autooxidation [437, 438], and the glucosamine pathway (O-linked glycosylation of proteins) [406, 439]. Activation of JNK and NF- κ B is also stress-induced [420, 423, 440]. The activated JNK phosphorylates the Ser³⁰⁷ residue of IRS-1, hence blunting IRS signaling leading to decreased nuclear PDX-1 [420]. The IRS proteins are intracellular tyrosine kinase substrates that are downstream of their receptor. The IRS-1 and IRS-2 are important for β -cell function and survival; their absence leads to insulin resistance [441–443]. The insulin-insulin receptor (IR)-IRS-PI3K-Akt signaling cascade is crucial for regulating islet cell differentiation and function. Decreased Akt signaling as a result of IRS-1 phosphorylation by JNK results in an increase in Foxo-1-dependent gene expression, which plays a role in mediating PDX-1 translocation from the nucleus [444].

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that controls anabolic cellular processes in response to a variety of environmental stimuli including amino acids, glucose and oxidative stress [445, 446]. The rapamycin-sensitive complex TORC1 phosphorylates S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1). The rapamycin-insensitive complex TORC2 phosphorylates Ser473 of Akt and PKC [447]. Continuous activation of mTOR by glucose/fatty acids leads to IRS-2 phosphorylation, tagging it for proteasomal degradation, resulting in decreased IRS-2 levels and increased β -cell apoptosis [448]. As discussed earlier, GLP-1 has a growth-promoting effect on β -cells and enhances proliferation and protects against apoptosis [411]. The GLP-1 receptor activation in β -cells leads to IRS-2 and PKB activation mediated by CREB and transactivation of EGFR [449].

Constant entry of glucose into the β -cell leads to a state of reversible insensitivity to glucose stimulation concomitant with an exhaustion of β -cell stores. When glucotoxicity occurs, β -cell damage leads to defects in insulin production such that chronic glucose exposure-induced β -cell exhaustion becomes irreversible. Some of the explanations for glucose-induced β -cell damage include depressed rates of insulin synthesis [450], increased small heterodimer partner (SHP) nuclear receptor mRNA [451], downregulation of glucokinase mRNA [425], reduced PDX-1 mRNA, protein and insulin promoter binding activity [312, 450, 452], compromised mitochondrial function and induction of apoptosis. Upregulation of the SHP receptor is thought to act as a competitive inhibitor to prevent p300-mediated PDX-1 and BETA2 complex formation [451]. Repeated exposure to elevated glucose also reduces activity of RIPE3b1 [453, 454], while enhancing activity of insulin transcriptional repressor basic leucine zipper CCAAT/enhancer-binding protein β (C/EBP β) [455, 456]. Changes in PDX-1 expression and activity have a profound influence on insulin transcription. Supra-physiological concentrations of glucose in the β -cell over an extended period of time are thought to affect posttranscriptional processing of PDX-1 mRNA [452]. The effect of short-term excess levels of glucose may be a reversible glucose desensitization whereas chronic, repeated exposure (long-term) causes less reversible effects on β -cell

function, particularly in regards to insulin synthesis [450]. The effect on β -cells is most likely due to effects on function rather than simply apoptosis, per se [405]. For example, patients that undergo 60 % pancreatectomies do not always develop hyperglycemia and are able to compensate by enhanced functionality in remaining β -cells. Hence, initial pathology of T2D most likely involves an initial defect in β -cell responsiveness to glucose, leading to reductions in β -cell mass.

Lipotoxicity

The effect of fatty acids on β -cell apoptosis is complex and is most likely due to a multitude of factors including ceramide formation, oxidative stress, unfolded protein response, and the inflammatory response as reviewed in [415, 416]. The effects of chronic exposure to fatty acids are a bit less clear-cut but appear to involve increased lipolysis in white adipose tissue as a result of insulin resistance, a process that is amplified with body weight gain and continuous accumulation of adipose tissue [405]. This destructive cycle results in elevated circulating levels of free fatty acids that have a negative effect on GSIS and exacerbate insulin resistance in muscle and liver cells [405, 408]. Saturated fatty acids in particular, such as palmitate and oleate, have inhibitory effects on GSIS [406]. Additionally, accumulation of adipose tissue leads to increased adipocyte secretion of cytokines and adipokines such as TNF- α , IL-6, leptin, resistin and adiponectin [457]. These cytokines can be cytotoxic to β -cells, especially TNF α [458]. Although elevated levels of free fatty acids can mediate cytotoxic effects in β -cells, it is likely that in the absence of hyperglycemia and/or pre-existing β -cell functional defects, these signals may play a role in adaptation to insulin resistance without necessarily having deleterious consequences to cell viability [406].

Glucolipotoxicity -Inhibition of β -oxidation, stimulation of complex lipid formation, and mitochondrial and ER stress

The effects of glucolipotoxicity may stem from the effects of glucose on fatty acid metabolism in β -cells [421, 459]. In the context of “glucolipotoxicity”, glucose metabolism in the β -cell leads to formation of citrate, a signal for formation of malonyl-CoA in the cytosol, which then inhibits carnitine palmitoyl transferase-1 (CPT-1) activity. This has the effect of blocking fatty acid oxidation since CPT-1 plays a key role in transporting fatty acids into the mitochondria, the site of β -oxidation. This leads to accumulation of long-chain acyl-CoA esters in the cytosol. Consequently, detoxification of fat is attenuated and free fatty acids are shunted into pathways that lead to formation of cytotoxic complex lipids [145, 406, 459], through activation of AMP-activated kinase (AMPK), a metabolic sensor that drives energy metabolism [460–464]. Activity of AMPK is inversely correlated with glucose concentration and is enhanced by fatty acids in β -cells. Activation of AMPK leads to lipogenesis via transcription factor sterol-regulatory-element-binding-protein-1c (SREBP1c) [465]. Importantly, the form of lipid has a profound effect on β -cell function. Triglycerides (TGs) are relatively non-toxic, monounsaturated fatty acids are protective due to their propensity for esterification into TGs, HDLs are protective, while saturated fatty acids such as palmitate, as well as oxidized LDLs induce cell death [415, 466–468].

One of the reasons that it has been difficult to ascribe β -cell dysfunction to a single factor is that glucolipotoxic conditions lead to multiple metabolic pathways with various metabolites and intermediates, each having different effects on cellular metabolism [415]. The mechanism of action of fatty acids in β -cells is debatable due to the ability of fatty acids to either directly cross the lipid-bilayer and act intracellularly or by their ability to activate the cell-surface receptors such as GPR40 [415]. In general, repeated exposure of β -cells to fatty acids dampens GSIS, reduces nuclear translocation of PDX-1 and expression of MafA, down-regulates insulin expression and induces apoptosis [79, 415, 469–472]. Although

exact mechanism by which chronic exposure of β -cells to saturated fatty acids leads to impaired insulin secretion is unclear, there are a variety of changes observed, including upregulation of the mitochondrial inner membrane protein uncoupling protein 2 (UCP2) [420, 473, 474], activation of PLC- ϵ [475], changes in insulin granule secretory machinery [476], and a dissociation of insulin secretory granules from voltage-gated Ca^{2+} channels [477].

The increased flux of glucose and fatty acids places a tremendous burden on mitochondrial oxidation, leading to increased membrane potential as well as ROS production [478]. As discussed earlier, the β -cell has a limited ability to cope with oxidative stress due to inherently low expression of antioxidant enzymes. The activation of UCP2 by ROS can dissipate the membrane potential by allowing protons to leak into the mitochondrial matrix, and couple the oxidation of fuel to heat rather than ATP [478]. In islets from human donors with T2D as well as *ob/ob* mice, UCP2 was up-regulated [479, 480]. Although this mechanism has a protective effect against generation of ROS, the reduction in ATP production and hence decreased ATP/ADP ratio leads to reduced insulin-secretory capacity [478, 481]. This places the mitochondria in a critical position for regulating cell function since glucose sensing requires production of ATP from oxidative phosphorylation [482]. Deletion of the UCP2 gene as well as reduction of endogenously produced superoxide in the mitochondria restored islet ATP levels and enhanced GSIS [480, 483].

Since apoptotic pathways converge in the mitochondria with caspase-3 activation and cytochrome C export, function of this organelle may be key to the orchestration of events leading to cell death under conditions of glucolipotoxicity [484]. It was observed that C57BL/6J mice fed a high-fat diet for 12 wk showed a 60 % increase in mitochondria mass (despite no change in number of mitochondria) [484]. Strains of mice with a 5-exon deletion in nicotinamide nucleotide transhydrogenase (*nnt*) show impaired glucose clearance, and lack of GSIS [485, 486]. This enzyme is an important component of the respiratory chain, converting NADP^+ and NADH into NADPH and NAD^+ , respectively. It is suggested that ROS/aging-associated increases in mitochondrial DNA (mtDNA) mutations could lead to increased susceptibility of the β -cell to metabolic overload [487].

ER stress and the unfolded protein response (UPR) are also implicated in β -cell dysfunction [302]. The high demand for insulin secretion as a result of chronic glucose and fatty acid-induced signaling places a tremendous metabolic burden on the ER in β -cells. The key players in the ER stress response include PERK, interferon response element (IRE)-1/X-box binding protein (XBP)-1 and activating transcription factor (ATF)-6 [488, 489]. The initial goal of the UPR is to activate chaperones such as Bip/GRP78 and GRP94 (heat shock protein 90; HSP90) and folding enzymes such as protein disulfide isomerase and peptidyl-prolyl *cis-trans* isomerase [489]. These proteins prevent aggregation of unfolded proteins and promote increased fidelity of protein folding. The ATF6 moves from the ER to Golgi where it is cleaved to release its bZIP domain, which then migrates into the nucleus and induces transcription of proteins involved in protein folding and ER-associated protein degradation [490]. To reduce the burden on the ER, protein translation is temporarily halted save for select proteins. The PERK, through phosphorylation of eIF2 α mediates a reduction in ER load and an increase in translation of the bZIP transcription factor ATF4, leading to an increase in transcription of C/EBP homologous protein (CHOP; GADD153) and GADD34, which aid in cell recovery [491, 492]. The IRE1 is a kinase/endoribonuclease that splices XBP-1 mRNA, which is then translated into a bZIP transcription factor that induces transcription of protein-folding-related genes [491, 493]. Misfolded proteins are targeted for degradation by ubiquitination in the cytosol. In the event that this response is insufficient to attenuate the accumulation of misfolded proteins and ER function is compromised, apoptosis is induced. Thus, The PERK is responsible for the initial response of temporarily

halting protein translation and entry into the ER to prevent overloading [491]. The IRE1 and ATF6 enhance transcription of genes encoding proteins that mediate protein folding, export and degradation.

To summarize the effects of glucolipotoxicity, high glucose levels prevent metabolism of fatty acids which results in funneling to pathways involving formation of toxic compounds (e.g., ceramide), which in turn down-regulate insulin, cause β -cell dysfunction, and results in apoptosis [420]. The chronic exposure of cells to glucose and fatty acids places a tremendous metabolic burden on the mitochondria and ER. Production of ROS in the mitochondria, up-regulation of UCP2 leading to decreased ATP production, and induction of the unfolded protein response may all be central to the series of events leading to apoptosis.

GENOME-WIDE ASSOCIATION STUDIES

The sequencing of the human genome, combined with advances in DNA sequencing technology allowed genome-wide association studies (GWAS) to emerge as a powerful technique for understanding the genetic basis of T2D [494]. Over the past 5 years, the field has grown exponentially, with at least 44 candidate gene loci identified that show significant associations with T2D [494]. This research has allowed for substantial progress in diabetes research because it has shed light on the physiological processes that are disrupted in the pathogenesis of diabetes, which has allowed for development of therapeutic strategies. A number of T2D candidate genes are linked to pancreatic beta cell function, providing strong evidence that alterations in insulin synthesis and secretion are central to the development of the disease [495]. The *KCNJ11* (potassium inwardly-rectifying channel, subfamily J, member 11) is a common variant that was identified in 2003, and encodes a zinc transporter restricted to beta cells that is a target of sulphonylureas, drugs that bind ATP-dependent potassium channels on beta cells leading to depolarization and insulin secretion [494, 496]. Other genes with variants associated with reduced insulin secretion in diabetic patients include *TCF7L2* ([497]; transcription factor 7-like 2), *SLC30A8* ([498, 499]; solute carrier family 30; zinc transporter, member 8), and *CDKAL1* ([500]; CDK5 regulatory subunit associated protein 1-like 1) [495]. The effect size for individual and combined loci is small, indicating that environmental and epigenetic factors play a large role in disease development in patients with genetic predisposition to the disease [494].

CONCLUSIONS AND IMPLICATIONS

In conclusion, insulin is a key metabolic hormone and defects in production, secretion and viability of pancreatic β -cells lead to diabetes mellitus, a disease of epidemic proportions in the American population. Insulin production is regulated at the transcriptional level by a number of transcription factors and post-transcriptionally by mRNA stability and factors that influence rates of protein translation. Insulin production and secretion pathways are responsive to nutrients, hormones and other environmental factors, with secretion in healthy individuals fine-tuned to match the metabolic needs of the body. Pancreatic β cell apoptosis is a hallmark of both T1D and T2D although the mechanisms leading to islet destruction are different. Auto-immune reactions leading to the destruction of insulin-producing cells play a key role in T1D. Excess levels of circulating glucose and fatty acids, termed “glucolipotoxicity”, is central to the pathogenesis of T2D, leading to pancreatic β -cell dysfunction. The metabolic changes involved are likely mediated through oxidative activity in the mitochondria and abnormal protein folding in the endoplasmic reticulum. Excess ROS and accumulation of unfolded proteins eventually lead to apoptosis. Islet destruction hinders compensatory insulin secretion in insulin resistant individuals and the disease progresses to T2D. The molecular mechanisms leading to both T1D and T2D are complex and not completely understood. Both forms of diabetes converge with loss of β cell mass. Hence,

therapeutic strategies targeted at preserving and enhancing β -cell mass are likely to be important as this disease continues to grow in prevalence.

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ABBREVIATIONS

AGEs	Advanced glycation end products
AMPK	AMP-activated kinase
ATF	Activating transcription factor
CAMPS	cAMP sensor
ClC-3	Chloride channel -3
CPT-1	Carnitine palmitoyl transferase-1
CRE	cAMP response element
CREB	cAMP response element binding protein
DAG	Diacylglycerol
DHAP	Dihydroxyacetone phosphate
DHP	Dihydropyridines
ER	Endoplasmic reticulum
FFA	Free fatty acid
FFAR-1	Free fatty acid receptor -1
G6P	Glucose-6-phosphate
GABA	Aminobutyric acid
GH	Growth hormone
GHR	Growth hormone receptor
GIP	Insulinotropic polypeptide
GISS	Glucose-induced insulin release
GLP-1	Glucagon-like peptide-1
GLUT2	Glucose transporter 2
Gly3P	Glycerol-3-phosphate
GPx	Glutathione peroxidase
GS-3	Glycogen synthase kinase
GSH	Glutathione
GSSG	Oxidized GSH
gSUR	Granule SUR
GWAS	Genome -wide association studies

HCSP	Highly Ca ²⁺ -sensitive pool
HLH	Helix-loop-helix domain
HVA 1	High voltage-activated
IAPP	Islet amyloid polypeptide
IBMX	3-isobutyl-1-methylxanthine
ICA512	Islet cell autoantigen 512
IFN- γ	Interferon- γ
IGF-1	Insulin-like growth factor-I
IL-1β	Interferon-1 β
iNOS	Inducible nitric oxide synthesis
IP3	Inositol 1,4,5-trisphosphate
IRE	Interferon response element
IRS-2	Receptor substrate
JNK	c-Jun NH2-terminal kinase
K_{ATP} channel	ATP-sensitive potassium channel
LVA	Low voltage-activated
MAPKs	Mitogen-activated protein kinases
MCP-1	Monocyte chemoattractant protein-1
NF-kB	Factor nuclear factor -kB
NO	Nitric oxide
OAA	Oxaloacetate
ODNs	Oligodeoxynucleotides
PDE	Phosphodiesterase
PERK	Pancreatic eukaryotic translation initiation factor 2-alpha kinase
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein kinase A
PKB/Akt	Protein kinase-B
PKG	cGMP/cGMP-dependent protein kinase
PP1	Protein phosphatase 1
PTBPs	Polypyrimidine tract binding proteins
REM	Ras exchange motif
rER	Rough endoplasmic reticulum
Rim2	Rab3-interacting molecule 2
RIPE3b	Rat insulin promoter element 3b
ROS	Reactive oxygen species

RRP	Readily releasable pool
SERCA-2b	Sarcoendoplasmic reticulum Ca ²⁺ ATPase type 2b
SHP	Small heterodimer partner
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SOD	Superoxide dismutase
SREBP1c	Sterol-regulatory-element-binding-protein-1c
SRP	Signal recognition particles
TGs	Triglycerides
T2D	type 2 diabetes
TNF-α	Tumor necrosis factor- α
TSS	Transcription start site
UCP-2	Uncoupling protein-2
XBP	X-box binding protein

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Fig. 1.
Schematic illustration of nutrient-regulated insulin secretion.

Fig. 2.
Schematic illustration of pancreatic beta-cell apoptosis and dysfunction.

Fig. 3.
Schematic illustration of immunological aspect of T1D.