The dynamic plasticity of insulin production in β-cells

Brandon B. Boland, Christopher J. Rhodes, Joseph S. Grimsby

ABSTRACT

Background: Although the insulin-producing pancreatic β-cells are quite capable of adapting to both acute and chronic changes in metabolic demand, persistently high demand for insulin will ultimately lead to their progressive dysfunction and eventual loss.Recent and historical studies highlight the importance of ‘resting’ the β-cell as a means of preserving functional β-cell mass.

Scope of Review: We provide experimental evidence to highlight the remarkable plasticity for insulin production and secretion by the pancreatic β-cell alongside some clinical evidence that supports leveraging this unique ability to preserve β-cell function.

Major conclusions: Treatment strategies for type 2 diabetes mellitus (T2DM) targeted towards reducing the systemic metabolic burden, rather than demanding greater insulin production from an already beleaguered β-cell, should be emphasized to maintain endogenous insulin secretory function and delay the progression of T2DM.

Keywords T2DM; Insulin production; β-cell rest

1. HOMEOSTASIS I: RAPID INTRACELLULAR CHANGES MEET METABOLIC DEMAND

1.1. Nutrient sensing

Insulin-producing pancreatic β-cells are metabolically bound to the maintenance of physiological glycemic homeostasis. The acute response of the β-cell to elevations in circulating glucose concentrations following a meal has been extensively studied. However, the unique ability of the β-cell to adapt to chronic changes in glycemia conferred by a range of caloric extremes, from starvation to over-nutrition, has been underappreciated yet reveals a remarkable adaptive plasticity of the β-cell to produce insulin depending on metabolic need. In this article, we examine the functional consequences of altered (pro)insulin biosynthesis and secretion in response to both acute and chronic changes in metabolic homeostasis.

The primary regulator of insulin secretion in β-cells is the circulating plasma glucose concentration [1]. The β-cell is fundamentally poised to sense glucose in the physiologically relevant range. It is equipped with the plasma membrane glucose transporter GLUT2 as well as glucokinase, which both have a Km for glucose in the millimolar range to sense glucose in the physiologically relevant range. It is equipped with the plasma membrane glucose transporter GLUT2 as well as glucokinase, which both have a Km for glucose in the millimolar range complementary to sensing circulating glucose concentrations. Once glucose enters the cell via GLUT2, it is phosphorylated by glucokinase to form glucose-6-phosphate in the rate limiting step of this process.

The low expression of the high affinity forms of hexokinase in β-cells, which display end-product inhibition coupled with the sigmoidal enzyme kinetic behavior of glucokinase (nH = 1.7), ensures that at low glucose concentrations (<2.5 mM) there is little substrate phosphorylation leading to greatly diminished glycolytic flux and reduced ATP/ADP ratio [2,3]. Once formed, glucose-6-phosphate enters glycolysis to form pyruvate, which enters the mitochondrial Krebs’ cycle to produce NADH and FADH2, which can then be oxidatively metabolized to produce ATP. β-cells have a unique metabolic inventory, not only geared toward ATP production but also for the generation of metabolic stimulus-coupling signals to regulate insulin production and secretion. For example, they lack both lactate dehydrogenase and the monocarboxylate transporter as an insurance policy against glucose-independent exercise-induced insulin secretion that could potentially result in fatal hypoglycemia [4]. Increased glycolytic flux in β-cells consequently increases the ATP/ADP ratio that then causes plasma membrane ATP-sensitive K+ channels to close [5], leading to depolarization and subsequent opening of voltage-dependent L-type Ca2+ channels (VDCCs) and rapid influx of Ca2+ into the cell. The rise in intracellular cytosolic Ca2+ is the primary mediator of insulin exocytosis [6], as it arbitrates the necessary events including insulin secretory granule (β-granule) traffic to the plasma membrane, priming, docking, and exocytotic regulated release via SNARE complexes.
Although glucose is the primary mediator of insulin secretion, a variety of other factors and macromolecules can contribute. Amino acids, fatty acids, incretins, some neurotransmitters, and certain pituitary hormones can influence insulin secretion. Growth hormone and prolactin both stimulate insulin secretion [7], the latter of which has also been shown to promote islet hypertrophy and hyperplasia during pregnancy. Interestingly, the prolactin inhibitor bromocriptine is part of the modern arsenal to combat T2DM; however, its anti-diabetic efficacy is more likely mediated by dopaminergic action rather than effects on prolactin-mediated insulin release [8]. Cortisol inhibits insulin secretion [9], while oxytocin was found to increase insulin secretion from isolated mouse islets in a glucose-independent manner [10]. Activation of membrane fatty acid receptors on the β-cell has contrasting effects on insulin secretion. The activation of the long chain fatty acid receptor GPCR40 has been shown to potentiate insulin secretion via phospholipase C activation [11], while activation of short chain fatty acid receptors FFAR2/FFAR3 may antagonize regulated insulin secretion [12,13]. β-cell metabolism of endogenous fatty acids to long chain acyl-CoA can potentiate glucose stimulated insulin secretion, similar to malonyl-CoA [14], but the high glucose levels required for insulin secretion inhibit β-oxidation to some extent. Some amino acids, including the combination of L-glutamine and L-leucine, or L-arginine alone can contribute to insulin secretion by raising intracellular Ca$^{2+}$ levels, albeit through different mechanisms [15–18]. Beta-cells also contain neurotransmitter receptors on their cell surface, and considering the high degree of innervation present in pancreatic islets, it is not surprising that the nervous system has a profound effect on insulin secretion. For instance, the reduced fasting glycemia in splanchnicectomized dogs suggests an inhibitory role for the sympathetic nervous system on insulin secretion [19], which is further corroborated by the observed increase in basal insulin secretion observed in pancreatic transplant recipients [20]. Sympathetic signals such as epinephrine inhibit insulin secretion [21], while parasympathetic signals such as acetylcholine potentiate glucose stimulated insulin secretion [22]. The observation that orally delivered glucose leads to a greater insulin secretory response compared to intravenously administered glucose laid the foundation for the hypothesis that the gut may secrete an insulinotropic factor [23]. Indeed, both GLP-1 and GIP are gut hormones secreted by enteroendocrine cells in the intestine in response to macronutrients. In addition to potentiating glucose-stimulated insulin secretion, these peptides have garnered substantial pharmaceutical attention, particularly GLP-1 [24]. The mechanism underlying the potentiation of insulin secretion was found to be Ca$^{2+}$-dependent; both PKA and, to a lesser extent, EPAC activation contribute to the dose-dependent increase in glucosed-stimulated secretion [25,26]. However, chronic hyperglycemia has been implicated in PKA-mediated GLP-1 receptor down-regulation, which may contribute to the blunted incretin effect observed during T2DM [27]. Recently, GLP-1 receptor agonism with physiological concentrations of GLP-1 (pmol/L) was found to activate PLC and, subsequently, the TRP family of channels leading to Na$^{+}$-mediated depolarization and insulin secretion [28,29]. Furthermore, GLP-1 has been shown to reduce β-cell apoptosis [29], delay gastric emptying [30], and inhibit food intake at the level of the arcuate nucleus [31]. The range of factors known to influence insulin secretion highlights the importance of the β-cell in maintaining organismal homeostasis by rapidly responding to metabolic changes. In this sense, the fate of insulin secretory granules (referred to as β-granules herein) within the β-cell is reflective of its external environment and should be considered when examining the plasticity of this endocrine cell.

### 1.2. Parallel (pro)insulin biosynthesis

Under normal circumstances, the β-cell retains a remarkable state in which insulin lost from intracellular stores by stimulated β-granule exocytosis is rapidly replenished by a parallel and specific upregulation of proinsulin biosynthesis (and other key β-granule proteins) [30]. One might assume that the regulation of insulin secretion and proinsulin biosynthesis are controlled similarly (although the molecular mechanisms are obviously different, i.e., regulated exocytosis versus protein synthesis translational control) [33]. However, there are some notable differences. The glucose threshold required for the secretion of insulin is between 4 and 6 mM, while the threshold for the biosynthesis of proinsulin is only 2–4 mM [34]. This bias assures (pro)insulin production occurs even in the absence of glucose-stimulated insulin secretion to ensure an optimal reservoir of insulin secretory stores. Glucose-induced proinsulin biosynthesis is Ca$^{2+}$-independent and is regulated instead by alternative metabolic secondary signals produced from β-cell mitochondria in response to nutrient metabolism [35]. In particular, acute elevations in glucose following a meal induce rapid translation of preproinsulin mRNA. Other macronutrients, such as fatty acids, do not stimulate proinsulin biosynthesis despite being potent secretagogues for insulin secretion. Indeed, under conditions of chronic hyperlipidemia, excess fatty acids can lead to the depletion of intracellular insulin stores [36]. Similarly, the sulfonlurea class of drugs, including glibenclamide and glyburide, are powerful secretagogues but do not promote compensatory proinsulin biosynthesis [37]. Furthermore, they may accelerate β-cell loss, as ex vivo treatment of human islets with sulfonlureas lead to a nearly 3-fold increase in apoptosis [38]. The ADOPT study revealed ephemeral glycemic control in type 2 diabetics receiving glyburide monotherapy; glucose began to rebound only 3 months following treatment initiation [39]. As such, although sulfonlureas improve HbA1c in the short term, over time they are detrimental to β-cell function as they severely deplete insulin secretory capacity [40]. Acetylcholine does not appear to control insulin biosynthesis, but norepinephrine markedly inhibits both glucose-stimulated insulin secretion and production [22]. Finally, proinsulin biosynthesis is not regulated by the autocrine action of insulin [41]. A summary of factors with known effects on insulin secretion and (pro)insulin biosynthesis is provided in Table 1.

The regulation of proinsulin biosynthesis is predominately regulated at the translational level to allow a rapid and dynamic response to glucose that efficiently replenishes insulin secretory stores. This specific regulation of proinsulin biosynthesis by glucose also applies to ~50 other β-cell proteins, all of which are β-granule proteins [122,123]. As such, it is the principle mechanism for controlling β-granule biogenesis. This specific translational control was found to be orchestrated by a unique stem-loop in the 5’ UTR of preproinsulin mRNA [124], which is common to other β-granule protein mRNAs as well [118,123,125]. Translational control requires stimulus-coupled mitochondrial metabolism of glucosed independent of β-cell depolarization and Ca$^{2+}$ [126,127]. Longer term (8 h) glucose administration can stabilize preproinsulin mRNA through an UGAA sequence and the PTB domain on the 3’ UTR [124]. Even longer term (18 h) glucose exposure regulates preproinsulin gene expression [128], but these are relatively small effects and are not relevant under normal circumstances where fluctuations in glucose occur approximately 2 h post-prandially. Notwithstanding, the predominant translational control of proinsulin biosynthesis by glucose ensures that insulin secreted via exocytosis is rapidly replenished under normal circumstances to maintain intracellular insulin stores at optimal levels. The production of insulin occurs in multiple, well-characterized steps. First, a preproinsulin precursor is translated, which contains an N-
A summary of nutrients, peptide hormones, ions, neurotransmitters, and pharmaceuticals with known effects on β-cell proinsulin biosynthesis and insulin secretion.

| Effector | Proinsulin Biosynthesis | Insulin secretion | Remarks | Reference |
|----------|-------------------------|-------------------|---------|-----------|
| **Nutrients** |                         |                   |         |           |
| Glucose  | Stimulates              | Stimulates        | Primary metabolic stimulus | [42,43] |
| Mannose  | Stimulates              | Stimulates        | Similar to glucose stimulation | [42,43] |
| Mannitol  | Inhibits                | Inhibits          | Inhibits glucose stimulation by GK inhibition | [42,43] |
| Fructose | No effect               | Stimulates        | Potentiates glucose stimulation | [42–45] |
| Xylitol  | No effect               | No effect         |         | [34,46]   |
| Ribose   | No effect               | No effect         |         | [46,47]   |
| Galactose| No effect               | No effect         |         | [43,48]   |
| N-acetylgalactosamine | Slight stimulus | Stimulates |         | [43,49]   |
| Other glucose | No effect | No effect | Includes allose, altrose, gulose, idose, talose | [50]   |
| Other sugars | No effect | No effect |                   |           |
| Dihydroxyacetone | Slight stimulus | Stimulates | Potentiates glucose stimulation | [42,52] |
| Pyruvate  | Slight stimulus         | Slight stimulus  | Methyl-ester form | [32,55]   |
| Succinate | Stimulates              | Stimulates        | Methyl-ester form | [56–58]   |
| Fumarate  | No effect               | No effect         | Methyl-ester form | [56,57]   |
| Citrate   | No effect               | No effect         | Methyl-ester form | [56,57]   |
| α-Ketosaccharide | Stimulates | Stimulates | Potentiates glucose stimulation | [52,53,59] |
| Leucine   | Stimulates              | Stimulates        | Only in the presence of glucose | [52,59]   |
| Glutamine | No effect               | No effect         |       | [32,60]   |
| Arginine  | No effect               | Stimulates        | Possibly stimulates insulin secretion by depolarization | [16,61,62] |
| Insosine  | Slight stimulus         | Slight stimulus  |         | [42,47,63] |
| Guanosine | Slight stimulus         | Slight stimulus  |         | [47,63]   |
| Adenosine | Slow stimulus           | Slow stimulus    |         | [63,64]   |
| Ketone bodies | Slow stimulus | Stimulates |     | [52,65,66] |
| Long Chain Fatty | No Effect | Stimulates |                   | [65,67]   |
| Short Chain Fatty | No Effect | Inhibit/Stimulate | Conflicting reports in the literature | [65,68–72] |
| **Peptide hormones** |                         |                   |         |           |
| ACTH      | Stimulates              | Stimulates        |         | [73,74]   |
| Corticosterone | Inhibits | Inhibits |                   | [75,76]   |
| Growth Hormone | Stimulates | Stimulates |         | [74,77,78] |
| Glucagon  | Stimulates              | Stimulates        | In vitro studies - potentiates glucose stimulation | [79,80]   |
| GLP-1 (7–37) | Stimulates | Stimulates | Potentiates glucose stimulation | [81,82]   |
| GIP       | Stimulates              | Stimulates        | Potentiates glucose stimulation | [83] |
| Somatostatin | No effect | Inhibits | Inhibits glucose stimulated insulin secretion | [84] |
| IAPP      | No effect               | No effect         |       | [85–87]   |
| Type-1 interferons | No effect | No effect |                | [88,89]   |
| Inteleukin-1β | +/−                     | +/−                | Concentration dependent | [90–93] |
| Insulin   | No effect               | No effect         | No autocrine direct effect | [94,95]   |
| Prostaglan | No effect               | Stimulates        | Increases overall islet insulin synthesis via proliferative effect | [96–98]   |
| **Neurotransmitters** |                         |                   |         |           |
| Acetylcholine | No effect | Stimulates | Potentiates glucose stimulation | [89,100]   |
| Epinephrine | Inhibits | Inhibits | Inhibits glucose stimulation | [101–103] |
| Ions       |                         |                   |         |           |
| Ca²⁺      | Inhibits                | Stimulates        | Required for glucose stimulated insulin secretion. Inhibits general protein synthesis | [104–107] |
| Mg²⁺      | Stimulates              | Stimulates        | Stimulates insulin secretion by depolarization | [104,108,109] |
| Zn²⁺      | No effect               | Inhibits          | Required for proinsulin biosynthesis | [104,107] |
| **Pharmacologic** |                         |                   |         |           |
| Sulfonylureas | No effect | Stimulates |                   | [113,114] |
| Diazoxide  | No effect               | Inhibits          | Inhibits glucose stimulated insulin secretion | [115,116] |
| CaMP analogues | Stimulates | Stimulates | Potentiates glucose stimulation | [103,117] |
| Phorbol esters | Slight stimulus | Stimulates | Slight potentiation of glucose-stimulated proinsulin biosynthesis reported | [118–120] |
| Trifluoperazine | No effect | Inhibits |                   | [121] |

Terminal signal sequence enabling the newly formed proinsulin to enter the lumen of the rough endoplasmic reticulum (RER) to facilitate the proper folding of proinsulin, stabilized by three disulfide bonds [129]. The signal peptide of proinsulin is rapidly cleaved, likely cotranslationally, to form proinsulin. Proinsulin, the first prohormone to be discovered [130], is then trafficked from the RER through the Golgi apparatus continuum [131] and concentrates in restricted regions of the trans-Golgi network at sites where immature insulin granule formation occurs. Here, proinsulin to insulin and C-peptide processing begins by the action of two Ca²⁺-sensitive prohormone convertases, PCSK2 (PC2) and PCSK3 (PC1/3), with basic amino acid trimming of split-proinsulin intermediates by carboxypeptidase H/E [132,133]. Consistent with the internal pH of newly forming β-granules, PCSK2/PCSK3 display optimal activity at pH 5.5 [134]. This acidic pH optimum and the influx of Ca²⁺ into an immature β-granule initiate proinsulin processing and retain insulin accumulation within the organelle where it is stored.
In addition, it has been proposed that PCSK2 and PCSK3 are also regulated by granin chaperones, including 7B2 (PCSK3) and proSAAS (PCSK2), but only the former has an appreciable effect on facilitating proinsulin processing [138]. As well as Ca^{2+} influx, and as immature β-granules mature, there is also an influx of Zn^{2+} via zinc transporters, most notably ZnT10, allowing hexameric crystallization of insulin comprising six insulin molecules to two Zn^{2+} cations [137]. It should also be noted that proinsulin processing is sequential, where PCSK3 catalyzes the first cleavage event to produce the intermediate des-31,32 proinsulin [138], although both endopeptidases are capable of cleaving either site [139]. This sequential proinsulin processing is reflected in that des-31,32 proinsulin is the predominant component of the hyperproinsulinemia found circulating in type 2 diabetes [140].

1.3. The fate of β-cell insulin stores

Normally, the β-cell maintains an optimal intracellular storage pool of insulin granules that is not only preserved by the balance between (pro)insulin production and insulin secretion but also by longer term β-granule/insulin degradation mediated by autophagic mechanisms [122,141]. Like most neuroendocrine cells, β-cells have at least two secretory pathways, a regulated secretory pathway for the secretion of insulin in response to glucose, and a constitutive pathway that serves to renew membrane proteins and facilitate general non-regulated protein secretion and the delivery of newly synthesized plasma membrane proteins [142]. Radiolabeled pulse-chase experiments confirmed that (pro)insulin, regardless of whether it was newly synthesized or fully processed, is destined to the regulated pathway with 99% efficiency [143]. How proteins designated for secretion via the constitutive pathway or the regulated pathway are sorted at the trans-Golgi network remains unclear, as are the majority of the proteins that interact with (pro)insulin to direct its sequential synthesis, folding, trafficking, and packing. However, the rate-limiting step for proinsulin sorting is known to occur at the site of β-granule biogenesis in the trans-Golgi network, rather than the RER [144]. The specific proteins involved in (pro)insulin sorting at the Golgi likely involve a number of constituent proteins. Key proteins found to be essential for insulin trafficking at the trans-Golgi have been elusive, although the PICK1/ICA69 heterodimeric complex as well as SORCS1 have emerged as candidates [145,146]. The trafficking of new β-granules from the trans-Golgi requires kinesin-1, syntaxin-3, and TMEM24 [147–149], but non-protein components are also essential for regulated β-granule traffic. For example, Ca^{2+} is important for increasing β-granule transport to the plasma membrane in response to glucose, via Ca^{2+}-dependent activation of protein phosphatase-2Bβ to dephosphorylate and increase kinesin mediated β-granule transport [150]. The transit time from the trans-Golgi network to the plasma membrane can occur in as little as 30–40 min, and newly synthesized (pro)insulin can be preferentially secreted in as little as one hour following synthesis [143]. However, full conversion of proinsulin to insulin requires ~3 h [151], so during conditions of chronic hyperglycemia, there is an increased proportional secretion of incompletely processed insulin [140]. Also of note is that β-granules represent the richest sites of cholesterol accumulation in β-cells. Disruption of cholesterol biosynthesis leads to impaired membrane fusion and β-granule trafficking [152], while excessive cholesterol leads to grossly enlarged β-granules and disrupted trafficking [153]. As such, cholesterol mishandling of β-cells in type 2 diabetes could also contribute to the insulin secretory dysfunction.

Regulated insulin release is uniquely biphasic [154]. A few minutes following stimulation by glucose, the first phase of insulin secretion consists of a high amplitude but short duration burst of insulin exocytosis that largely consists of β-granules considered to be part of the readily releasable pool, that is, β-granules already docked and primed at the plasma membrane of the β-cell [155]. The reduced impedance of insulin secretion during the first phase is thought to be attributed to a number of proteins including gelsolin/syntaxin-4, which are involved in the unraveling of F-actin complexes near the plasma membrane, PKC dependent inactivation of the inhibitory Munc18/syntaxin-1 complex, and the synaptotagmin interaction with VAMP2 and SNAP-25 [156–160]. While most first phase insulin secretion consists of docked β-granules, some exocytosis of β-granules adjacent to the plasma membrane can also contribute. The trafficking of these β-granules is mediated by small GTPases, including Rab3A, Rab27, RabA, and Rap1, all of which aid in the docking and priming of these granules to the plasma membrane, potentially in a cAMP-dependent manner via EPAC activity [161,162]. The entire process of first phase insulin secretion is largely Ca^{2+} dependent [155], but the second phase of insulin secretion, which has a lower amplitude but longer duration [163], can contribute substantially to the total insulin output with prolonged stimulation [164]. The second phase of insulin release not only reflects exocytosis of primed and docked β-granules, but also reflects Ca^{2+}-dependent transport β-granules to the plasma membrane prior to undergoing exocytosis [150,163]. Often overlooked in the discussion of insulin exocytosis is the parallel endocytosis of the fused secretory granule membranes. If this did not occur, GSIS would lead to expanded β-cell size. While β-cell size does transiently increase (~4 min) following GSIS [165], early studies using electron microscopy and horseradish-peroxidase confirmed that β-cell size is stable over time and that the rate of exocytosis is equal to that of endocytosis [166].

Not all insulin granules undergo exocytosis, and if retained for ~5 days they are targeted for internal degradation by autophagy [122]. Normally, this system plays a janitorial role in maintaining optimal functional insulin secretory stores in the β-cell [167]. It has been presumed that this turnover occurs through macroautophagy (also referred to as crinophagy) [168], though β-granules are more commonly degraded by microautophagy, a process where aged β-granules are engulfed by autophagolysosomes [169,170]. In fact, bulk autophagy may be inhibited by the degradation of β-granules, as the resultant amino acids released from insulin catabolism would activate mTORC1 that, in turn, suppress macroautophagy [171]. As depicted in Figure 1, under normal circumstances, a large and rather stable number of mature insulin granules characteristically mark normal β-cell biology, and this population is maintained via equilibrium between secretion, biosynthesis, and degradation. However, if this balance is disrupted, such as in obesity or during a prolonged fast, the β-cell will adapt in compensation to prevent failures associated with aberrant insulin secretion or biosynthesis. These latter physiological circumstances are outlined in the following section.

2. Homeostasis II: Altered Metabolic Demand Challenges Glycemic Control

2.1. Response to starvation

The modern human genome was likely selected during the late Paleolithic era, when humans sustained themselves primarily as hunter-gatherers [172]. Early humans thrived in an environment characterized by intermittent periods of fasting and feasting. The selection of regulatory metabolic genes that protected against starvation by prioritizing food intake and storage was essential [173] for our survival. Many biological systems remain wired to quickly adapt to oscillations between nutrient abundance and scarcity, and the
pancreatic β-cell is no exception. Indeed, excessive insulin secretion during starvation could lead to detrimental hypoglycemia. Previous studies of β-cell ultrastructure provided clues as to how the β-cell adapts during starvation-refeeding. Prolonged starvation did not induce β-cell injury, but insulin stores become markedly reduced [174,175]. In normal rats fasted for 3 days, electron micrographs of 12-week old lean C57/B6 β-cells also reveal this massive insulin degranulation concurrent with increased numbers of autophagolysosomes and also an apparent expanded Golgi apparatus (unpublished data). This phenotype is portrayed in Figure 2, which shows the ‘poised’ state of the fasted β-cell, although depleted of mature β-granules to protect against hypoglycemia, the expanded Golgi stands ready for rapid (pro)insulin biosynthesis and maturation following a postprandial stimulus. Indeed, only 2–4 h after refeeding, β-cells replenish their stores of intracellular insulin and move towards a normal morphology (Figure 1), highlighting the ability of the entire cellular ultrastructure to rapidly and reversibly adjust to changes in metabolic demand (unpublished data). This remarkable adaptive plasticity is emphasized by insulinoma transplantation experiments in rats, where, in parallel to chronic hypoglycemia, endogenous β-cells ‘disappear’. However, following removal of the insulinoma, β-cells ‘reappear’ [176]. This finding cannot be explained by islet atrophy and subsequent regeneration but instead is the consequence of rapid endogenous β-cell degranulation in response to hypoglycemia (similar to that found in prolonged fasting), followed by prompt regranulation once circulating insulin levels are normalized and glucose homeostasis recovers. The β-cell adaptive plasticity can also be applied when the normal balance between insulin production and secretion becomes disrupted. An example is found in the lean Rab3a-/- transgenic mouse model.
Here, β-cells have reduced insulin secretion caused by deletion of the GTPase, Rab3A, necessary for β-granule transport, but (pro)insulin production remains normal. However, a normal cytoplasmic pool of β-granules is maintained by upregulating autophagy to rebalance the excessive biosynthesis of (pro)insulin relative to decreased insulin secretion in this model [168].

2.2. Response to overnutrition

Obesity is frequently characterized by insulin resistance, a presumed state of insufficient insulin production, and insulin secretory dysfunction despite high metabolic demand. In these circumstances, the β-cell can adapt by increasing β-cell mass through a variety of means, including hypertrophy, β-cell proliferation, neogenesis, and inhibition of apoptosis, all of which lead to enhanced insulin secretory capacity [177]. However, although the β-cell is beautifully geared to adapt to fasting/refeeding states, it seems to struggle to adjust to chronic overnutrition. If glucose levels remain persistently high, the β-cell becomes dysfunctional and is ultimately unable to sufficiently compensate. In this case, a variety of stressors related to hyperglycemia and increased insulin demand, including oxidative stress, endoplasmic reticulum (ER) stress, hyperlipidemic stress, amyloid stress, and inflammatory stress [178–181], have been proposed to contribute to β-cell dysfunction and ultimately apoptosis (see Section 3.2). In addition, the hyperinsulinemia associated with obesity-linked T2DM can further drive β-cell dysfunction by promoting insulin-induced insulin resistance [182]. Also, in an insulin resistant state, the desensitization of CNS insulin receptors could lead to a feed-forward loop that drives further β-cell dysfunction [183]. The characteristic consequences of β-cell dysfunction during T2DM include diminished first phase insulin secretion, increased basal insulin secretion, impaired glucose-sensing of the β-cell, and an increased proportion of proinsulin secretion [184,185]. However, it is unclear whether these manifestations of dysfunction contribute to the pathogenesis of T2DM, or if they are simply a consequence of β-cells striving to produce sufficient insulin to compensate for metabolic overload and an insulin resistant state [186,187]. Observations of reduced pancreatic insulin content in parallel to insulin insufficiency have led to the notion that β-cells during T2DM have impaired secretory capacity [188]. Nearly half a century ago, ultrastructural studies of β-cells following infusion of high glucose or anti-insulin antisera revealed massive insulin degranulation, implying a presumed defective insulin secretion [189]. Importantly, the long standing assumption that insulin production declines during β-cell dysfunction [184] has led to many treatment strategies aimed at increasing β-cell insulin secretion, most notably sulfonylureas and GLP-1 receptor agonists [190,191]. But long-term use of some of these, like sulfonyureas, eventually accelerate β-cell demise and dysfunction by compromising endogenous insulin secretory capacity [38,39,191].

3. AN UNPAID DEBT: CONTRIBUTING FACTORS TO β-CELL Dysfunction

3.1. The consequence of chronic metabolic demand

The observation that non-diabetic human obesity is associated with increased islet size has been known for 80 years [192]. Modern comparisons have confirmed that most obese non-diabetic donors have increased β-cell mass compared to lean controls, whereas pancreatic sections from obese T2DM, and even prediabetic subjects, have an apparent reduced β-cell mass [193–196]. Interestingly, the ability to adapt to metabolic demand may be conferred during the prenatal period, as a low birth weight was associated with reduced β-cell mass and an increased likelihood of development diabetes later in life [197]. The primary driver of β-cell mass adaptation in obesity is insulin resistance, which increases the demand for insulin [198]. Tissue-specific knockout of the insulin receptor, particularly in the liver, began to parse out the role of these metabolic tissues in insulin resistance and the need for consequential β-cell compensation [199–201]. However, despite the obvious importance of insulin resistance during obesity and T2DM, around two-thirds of obese individuals do not develop frank diabetes, suggesting that the β-cell is capable of adapting to metabolic demand to a degree in a majority of individuals [202]. Although numerous genetic susceptibility variants have been identified for T2DM, their effect sizes are too small to account for either the prevalence of the disease or its relatively recent emergence (~35 years) as a global epidemic [203–205], making it more likely that environmental factors such as diet and lifestyle are the predominant factors in the progression of T2DM. Once T2DM manifests in obese individuals, it is presumed that a stark decline in β-cell mass has been initiated. But this is difficult to demonstrate unequivocally, since there is currently no way to measure β-cell mass in living individuals, and it is estimated that most patients are not diagnosed until many years after the onset of T2DM [206]. This is indicated by there being significant β-cell loss in patients with impaired fasting glucose or pre-diabetes [195]. It is likely that rate of β-cell death via apoptosis as a consequence of stressors related to insulin resistance, inflammation, and hyperglycemia outweigh the rate of proliferation, as ex vivo studies of human islets revealed that glucolipotoxicity led to decreased markers of proliferation and increased apoptosis [207]. In this case, β-cell mass can become so depleted that a lifetime of supplementary insulin therapy would be the only recourse [208]. However, even as β-cell mass decreases, the remaining β-cells valiantly attempt to meet metabolic demand in the face of steadily increasing insulin resistance. Even in severely obese diabetic KS+db/db mice, that have an apparent decrease in β-cell mass, there is a marked increase in proinsulin biosynthesis in the remaining β-cells [209]. This upregulation of insulin production is facilitated by an expansion of the RER and Golgi apparaatus, an increase in the number of immature β-granules but a stark reduction in mature β-granules (the cell biology of such obese diabetic β-cells is illustrated in Figure 3). Yet these animals remain markedly hyperinsulinemic (≥10-fold higher than lean KS1/2 control mice [209]). So, it seems that these β-cells are producing as much (pro) insulin as they possibly can and then rapidly secreting it in a persistent effort to manage the hyperglycemia and compensate for insulin resistance, futile though that might be with such decreased β-cell mass in this mouse model [209]. Although isolated islets from these animals display all the hallmarks of secretory dysfunction in T2DM, including elevated basal insulin secretion, blunted first phase insulin secretion, an increase in the ratio of secreted proinsulin to insulin, and left-shifted glucose sensing, these ‘flaws’ could in fact all be part of the compensatory mechanisms to secrete more insulin to meet the metabolic demand in the face of insulin resistance. In this sense, these β-cells may not be dysfunctional, but rather functionally adapted to produce continuous large quantities of insulin.

3.2. A bombardment of stressors

A combination of many interrelated stressors causes β-cell loss in the pathogenesis of obese T2DM. As obesity develops, adipose tissue expands to provide a reservoir for the associated positive energy balance [210]. But, as a consequence, they release a variety of adipokines and proinflammatory cytokines as well as NEFAs and glycerol as a result of increased lipolysis [211]. This is in addition to increased dietary lipid intake and hepatic lipogenesis. The deleterious
A frequently attributed factor in secretory dysfunction and apoptosis of \( \beta \)-cells is ER-stress. When protein translation is increased, cells can compensate by activating the unfolded protein response (UPR). This process is mediated by at least 3 proteins, IRE1\( \alpha \), PERK, and ATF6, which collaborate to slow protein translation, increase the production of protein-folding chaperones and enhance membrane biogenesis [217]. The UPR can also be upregulated when foreign or variant proteins are synthesized, and if persistent can lead to ER-stress, which occurs primarily through CHOP induction, RER dilation, and subsequent apoptosis [218]. An excellent example of this is the ‘Akita mouse’ in which a proinsulin variant is synthesized and inappropriately folded, inducing ER-stress, \( \beta \)-cell apoptosis, and subsequent lean diabetes [219]. It has been estimated that up to 20% of newly synthesized proinsulin is misfolded, and since proinsulin accounts for 30–50% of the total \( \beta \)-cell translational load, it is not unreasonable to assume that the \( \beta \)-cell routinely manages a substantial amount of protein misfolding [220]. However, this notion has been recently challenged [201]. In \( \beta \)-cells of obese diabetic K\( \text{O}^{\text{db}} \text{db} \) mice, expanded RER and Golgi apparatus are observed to cater to increased proinsulin biosynthesis, but absolutely no evidence of a dilated RER or ER-stress was found [209]. The UPR is likely present to aid increased proinsulin production but not ER-stress, which would jeopardize this process [209]. Indeed, in light of massively upregulated insulin production in remaining \( \beta \)-cells in the pathogenesis of common obese T2DM, ER-stress is unlikely to play a significant role to \( \beta \)-cell loss.

Lipid peroxidation, protein oxidation, and DNA damage are all consequences of the excess generation of mitochondrial-derived products of oxidative phosphorylation. Oxygen is the final electron acceptor of respiration, the majority of which is reduced to \( \text{H}_2\text{O} \). However, a small percentage of the \( \text{O}_2 \) is converted to free radicals, such as superoxide anion (\( \text{O}_2^- \)), which are usually rapidly neutralized by a combination of cellular antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and catalase [221]. Pancreatic \( \beta \)-cells are wired to have high rates of ATP production not only to provide energy but also for secondary stimulus-coupling signals to stimulate insulin production and secretion. However, they paradoxically have low levels of some antioxidant enzymes, such as glutathione peroxidase and catalase [222]. As a consequence, \( \beta \)-cells are deficient in \( \text{H}_2\text{O}_2 \) clearance. Oxygen radical production has been implicated in disrupted insulin secretion but is also a necessary byproduct of ATP generation and subsequent GSIS [223]. Uncoupling proteins facilitate the return of protons to the mitochondrial matrix. UCP-2 in particular is induced by \( \text{O}_2^- \) and regulates the rate of respiration, mitochondrial membrane potential, ATP production, and insulin release [224]. Thus, it can protect \( \beta \)-cells from oxidative damage by slowing oxidative phosphorylation and attenuating GSIS [225]. However, this comes at the cost of reduced secretory capacity. Furthermore, ROS may disrupt insulin biosynthesis, as ROS exposed islets showed reduced insulin co-secreted with insulin and usually exists in a soluble form [214]. Like most other \( \beta \)-granule proteins, the biosynthesis of IAPP is coordinated with that of proinsulin [40]. Normally, IAPP functions centrally, where it contributes to postprandial satiety and gastric emptying. But when proinsulin, and proIAPP [40], biosynthesis are chronically increased in T2DM [208], IAPP can fold into cytotoxic aggregates, which have been implicated in \( \beta \)-cell apoptosis in primates [180]. IAPP aggregates form prior to the onset of frank T2DM during fasting hyperglycemia, implicating the onset of \( \beta \)-cell secretory dysfunction prior to IAPP aggregate deposition [215]. However, the occurrence of IAPP containing amyloid plaques is highly variable in islets from T2DM human cadavers (between 1% and 80%), suggesting that IAPP is not etiological in the pathogenesis of T2DM [216].

### Figure 3

A graphic representation and a transmission electron micrograph of a 12-week old obese diabetic KS \( \text{db/db} \) mouse displaying degranulation of mature insulin secretory granules, abundant immature granules, and an expansion of both the RER and Golgi apparatus. Magnification, 2500×.

**Representative \( \beta \)-cell from an obese diabetic KS \( \text{db/db} \) mouse displaying degranulation and abundant immature granules. Although phenotypically similar to a fasted \( \beta \)-cell, the lack of mature insulin granules is reflective of constant secretion in an attempt to meet glycemic demand. Here, both the Golgi and ER have expanded to compensate for increased (pro)insulin biosynthesis (2,500X).**

Effect of increased internal and external fatty acids on the \( \beta \)-cell is termed lipotoxicity. While fatty acids can potentiate insulin secretion, the absence of a complementary increase in proinsulin biosynthesis can deplete \( \beta \)-cells of mature insulin granules and contribute to dysfunction [166]. As obesity is accompanied by a chronic state of low-grade inflammation, enhanced secretion of proinflammatory cytokines, including TNF-\( \alpha \), IL-6, and IFN-\( \gamma \), is another consequence of increased adiposity [212]. In adipose tissue, these cytokines can create a vicious cycle as they lead to macrophage recruitment, greater inflammation, and subsequent increased cytokine release. These can then have detrimental effects on \( \beta \)-cells that influence insulin secretion and survival [213].

Factors intrinsic to the \( \beta \)-cell may play a more prominent role. One notable factor in humans, islet amyloid polypeptide (amylin, or IAPP) is

---

**Reviewer**

---

**References**

[180], [181], [209], [210], [211], [212], [213], [214], [215], [216], [217], [218], [219], [220], [221], [222], [223], [224], [225]
promoter activity and suppressed insulin mRNA activity [188], likely mediated by ROS-mediated activation of the c-Jun N-terminal kinase pathway (JNK) and subsequently reduced Pdx-1 binding to the insulin promoter [225]. As such, chronically elevated ROS production during obese T2DM in β-cells, likely driven by increased mitochondrial metabolism as a consequence of the chronic hyperglycemia and/or hyperlipidemia can cause oxidative stress leading to secretory dysfunction and apoptosis. The β-cells may be particularly susceptible to oxidative stress under such circumstances due to deficient expression of certain antioxidant enzymes [214].

3.3. Secretory dysfunction: beginning of the end?
In subjects with normal glucose tolerance and varying degrees of obesity, β-cell function was found to vary quantitatively with differences in insulin sensitivity [227]. In fact, studies in mice have revealed that β-cell secretory dysfunction is the primary driver of β-cell failure, rather than reduced β-cell mass, impaired glucose metabolism, or steatosis [228]. Several characteristic events mark the gradual loss of β-cell secretory dysfunction. Increased basal insulin secretion even in the absence of glucose stimulus is indicative of impaired glucose sensing [227]. Also, irregularity emerges in both the frequency and amplitude of the electrophysically-derived oscillations in insulin secretion [229]. In response to intravenous glucose administration, the amplitude of first phase insulin secretion is diminished, and, as the disease progresses, glucose-induced first phase insulin secretion is lost entirely [229,230]. In parallel, there is a delayed and blunted secretory response to mixed meal consumption, underlining the impaired incretin response observed in insulin resistant subjects [231]. The mechanism of the diminished incretin effect in T2DM is likely due to chronic hyperglycemia that blunts GLP-1 receptor signaling in β-cells via a PKA-dependent mechanism [27]. Furthermore, there is a greater proportion of secreted proinsulin in response to a meal during secretory dysfunction in T2DM [232]. It could be argued that the hyperinsulinemia present during β-cell secretory dysfunction may be partly the consequence of non-regulated constitutive secretion of incompletely processed insulin. However, although the amount of proinsulin secreted is increased, leading to an increased ratio of proinsulin to insulin in the circulation, the absolute levels of circulating proinsulin remain a small proportion of the total circulating insulin, in part due to a greater degree of basal insulin secretion in the absence of appropriate stimulation [233]. Fortunately, β-cell dysfunction does not necessarily guarantee β-cell demise. By lightening the chronic barrage of insults directed at β-cells, most notably persistently elevated glucose, β-cells can then rapidly regain functional secretory capacity. freshly isolated islets from either 6J or Kc [db/db] obese diabetic mice display hallmarks of secretory dysfunction and yet maintain a proinsulin biosynthetic rate that far exceeds that of their lean control counterparts. Further, when these “db/db islets” are allowed euglycemic recovery for 8–12 h, dysfunction is reversed and normal insulin secretory capacity and function return [209]. As such, the concept of ‘β-cell rest’ is re-emerging as a means to preserve functional β-cell mass and delay the progression of T2DM.

4. BEATING FORECLOSURE: STRATEGIES TO MITIGATE DEMAND AND RETAIN SECRETORY FUNCTION

4.1. Evidence for in vivo β-cell rest
Perhaps the most efficacious means of halting the progression of obesity-linked T2DM and islet dysfunction in most individuals is by reducing caloric intake to the point at which energy storage is no longer heavily prioritized. This has been clearly established in rodent models of advanced diabetes, where dietary restriction of db/db mice to 40% levels was found to preserve β-cell mass and function, in part, by reducing oxidative stress [234]. Likewise, in human individuals with T2DM, six-weeks of severe caloric restriction (>900 kcal/d) led to a doubling of their β-cell disposition index, which was likely a consequence of the 40% reduction in circulating insulin, indicative of reduced insulin demand [235]. In humans, lifestyle intervention including diet and exercise was found to be more effective than metformin for the prevention of T2DM [236]. Caloric restriction (CR) itself, particularly by alternating days of CR with ad libitum refeeding days, has shown potential to improve insulin sensitivity and reduce body weight. An 8-week study in obese individuals found that alternating days of 80% CR followed by ad libitum refeeding resulted in a 33% decrease in HOMA-IR and an 8% reduction in body weight [237]. All this might be viewed as in vivo evidence for taking effort off of the β-cell (i.e., ‘β-cell rest’) that then improves endogenous insulin secretory capacity. However, outside of an experimental setting, adherence to any form of caloric restriction for an extended period can be daunting for T2DM patients. Fortunately, a number of pharmaceutical strategies, particularly those that target multiple pathways independent of the β-cell, are attractive strategies to reduce the metabolic demand on the β-cell and preserve insulin secretory function.

More than 40 years ago, Greenwood et al. administered diazoxide to inhibit endogenous insulin secretion and promote a degree of transient ‘β-cell rest’ to healthy volunteers and T2DM patients, previously untreated with insulin. β-cell function was assessed using a glucagon stimulation and tolbutamide stimulation test prior to and after treatment. The excursions in endogenous insulin secretion following stimulation tests increased over baseline levels by 7%, 70%, 280%, and 500% in T2DM placebo, healthy subjects, T2DM (low β-cell reserve) and T2DM (high β-cell reserve) treatment groups, respectively [238]. The authors concluded that poor insulin response in diabetics resulted, in part, from chronic overstimulation, and the observed improvements following diazoxide treatment reflected an increase in endogenous β-cell insulin stores. Note that diazoxide inhibits insulin secretion but has no effect on (pro)insulin biosynthesis (Table 1). Similar diazoxide studies in T2DM human islets have also shown that such treatment can restore normal pulsatile insulin secretion by recovering insulin secretory capacity [239]. Glaser et al. found similar effects on improved β-cell function following short-term (16.6 ± 1.5 days), continuous subcutaneous insulin infusion in T2DM patients uncontrolled by conventional oral therapies. Near euglycemia was achieved (<7.7 mM glucose) throughout the treatment period, resulting in improved glycemic control after cessation of insulin infusion. β-cell function tests (glucagon stimulation and IV glucose) conducted 48 h following cessation of insulin infusion resulted in marked improvements in maximal incremental C-peptide response to glucagon and peak insulin response. The authors conclude “that chronic hyperglycemia induces a further (reversible) impairment in the already reduced insulin secretory capacity of the diabetic [240].” Taken together, these early observational data provided evidence that short-term improvement of glycemic control can be beneficial to β-cell function by inducing β-cell rest and/or decreasing glucotoxicity, but its contribution and impact on achieving optimal metabolic control was speculative due to limitations in the size and duration of the treatment. A Diabetes Outcome Progression Trial (ADOPT) evaluated rosiglitazone, metformin and glyburide as first line therapy for newly diagnosed T2DM patients in a double-blind, randomized, controlled clinical trial involving 4360 patients. The primary endpoint was monotherapy failure and the patients were treated for a median of 4 years [39]. Monotherapy failed
in 143 patients on rosiglitazone (2.9 per 100 patient-years), 207 on metformin (4.3 per 100 patient-years), and 311 on glyburide (7.5 per 100 patient-years). At 4 years of evaluation, 40%, 36%, and 26% of the patients in the rosiglitazone, metformin, and glyburide treatment groups, respectively, had %HbA1c < 7%. Rosiglitazone slowed the rate of β-cell dysfunction and improved insulin resistance to a greater extent compared to metformin and glyburide, which may explain the more durable effect on glycemic control. This also suggests that alleviating the demand on the β-cell by improving insulin sensitivity can induce a degree of β-cell rest that is beneficial to the treatment of T2DM. Conversely, the ADOPT study indicated that although glyburide had an initial beneficial effect, in the long run, it was detrimental to β-cell function and T2DM treatment [39]. This was likely due to accelerating reduced insulin secretory capacity of β-cells in T2DM, as although sulfonylureas increase insulin secretion, they have no effect on (pro) insulin production and deplete β-cell insulin stores (Table 1) [40].

4.2. Combination therapy and the case for early intervention

The ‘treat to failure approach’ may not be the optimal therapeutic strategy in slowing the progression of T2DM, and may do even less to retain residual β-cell function [241,242]. Failure implies deterioration of glycemic control and necessitates more aggressive intervention—a cycle that if allowed to continue unabated can lead to a lifetime of insulin dependence. Fortunately, this perception is changing, and if %HbA1c target goals are not achieved after initiation of metformin and lifestyle intervention, combination therapies are recommended and progress rapidly, approximately every 3 months (dual, triple and injectable therapy) until target goals are achieved. In 2009, the LEAD-4 study showed that the GLP-1 agonist lixivatide on top of rosiglitazone and metformin therapies in T2DM individuals was not only well-tolerated but led to a 1.5 point drop in %HbA1c, weight loss, and a reduction in FBG by 40 mg/dL. Most notably, HOMA-β, a mathematical surrogate for β-cell function, improved by 5-fold in patients receiving lixivatide in addition to the other therapies [243]. A study of T2DM patients on metformin found that 4-week treatment cessation from the GLP-1 agonist lixivatide following one year of combination metformin + exenatide therapy led to a massive decline in β-cell function back to pretreatment levels [244] that was comparable although slightly mitigated if treatment cessation occurred after 2 years of combination therapy [245]. Another study in 2013 utilized recently diagnosed T2DM patients and treated them with DPP-4 inhibition + T2D therapy or DPP-IV inhibition alone. Not only did the combination therapy show superior anti-diabetic efficacy in terms of weight loss, %HbA1c, and FBG, combination therapy induced robust improvements in β-cell function, as evidenced by improved β-cell glucose sensitivity and fasting secretory tone [246]. A meta-analysis of randomized controlled trials comparing SGLT-2 inhibition via canagliflozin in combination with metformin in T2DM patients found that HOMA2-%B was significantly improved by the addition of canagliflozin [247]. While all of these studies provide strong evidence for the efficacy of combination therapies in established T2DM patients, there is a scarcity of longitudinal data in patients at high risk of developing T2DM. Interestingly, a 10-year longitudinal study in 4106 participants with normal glucose tolerance found that impairment of β-cell function had a more profound effect on incident diabetes than did decreases in insulin sensitivity [248]. As such, these examples of clinical evidence are further supportive of the strategy to preserve endogenous β-cell functional mass for effective treatment of T2DM.

In 2017, the American Diabetes Association (ADA) suggested that prediabetes “...should not be viewed as a clinical entity in its own right but rather as an increased risk for diabetes [249].” As such, it is unsurprising that little clinical data exist on early pharmaceutical intervention in these at-risk individuals, even though a ‘non-diabetic’ HbA1c of 6.0% confers a 50% greater risk of developing diabetes than individuals with an HbA1c of 5.0% [250]. Considering that the β-cell is remarkably capable of restoring function in response to attenuated metabolic demand, an argument should be made for early intervention with a combination pharmaceutical approach to improve insulin sensitivity and/or reduce metabolic demand, that, in turn, creates less demand on the β-cell and preserves insulin secretory function to halt the onset or delay the progression of T2DM — thus following the aforementioned concept of β-cell rest. Key to this approach is early diagnosis of T2DM and early treatment to best maximize the return to normal function of what β-cell mass remains.

CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Ohmeda, M., Johnson, J.H., Inman, L.R., Unger, R.H., 1993. Glut-2 function in glucose-unresponsive beta-cells of dexamethasone-induced diabetes in rats. Journal of Clinical Investigation 92(4):1950–1956.
[2] Schuit, F.C., De Vos, A., Moens, K., Quartier, E., Heimberg, H., 1997. Glucose-induced β-cell recruitment and the expression of hexokinase iso-enzymes. Advances in Experimental Medicine and Biology 426:259–266.
[3] Delimy, P., Gilon, P., Henquin, J.C., 1998. Interplay between cytoplasmic Ca2+ and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets. The Biochemical Journal 333(Pl:2):269–274.
[4] Sekine, N., Cirulli, V., Regazzi, R., Brown, L.J., Gine, E., Tamarit Rodriquez, J., et al., 1994. Low lactate-dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells — potential role in nutrient sensing. Journal of Biological Chemistry 269(7):4895–4902.
[5] Inagaki, N., Goon, T., Clement, J.P., Nambo, N., Inazawa, J., Gonzalez, G., et al., 1995. Reconstitution of I-katp — an inward rectifier subunit plus the sulfonylurea receptor. Science 270(5239):1166–1170.
[6] Cury, D.L., Bennett, L.L., Grodsky, G.M., 1969. Requirement for calcium ion in insulin secretion by perfused rat pancreas. American Journal of Physiology 214(1):174.
[7] Engel, F.L., Albertson, T., Fredericks, J., Lopez, E., 1958. Evidence for stimulation of insulin secretion by growth hormone in the rat. Endocrinology 63(1):99–105.
[8] Defronzo, R.A., 2011. Bromocriptine: a sympatholytic, D2-dopamine agent for the treatment of type 2 diabetes. Diabetes Care 34(4):793–794.
[9] Plat, L., Byrne, M.M., Sturis, J., Polonsky, K.S., Mockel, J., Fery, F., et al., 1996. Effects of morning cortisol elevation on insulin secretion and glucose regulation in humans. American Journal of Physiology-endocrinology and Metabolism 270(1):E36–E42.
[10] Gao, Z.Y., Drews, G., Henquin, J.C., 1991. Mechanisms of the stimulation of insulin release by oxytocin in normal mouse islets. The Biochemical Journal 276(1):169–174.
[11] Shapiro, H., Shachar, S., Sekler, I., Hershflinkel, M., Walker, M.D., 2005. Role of GPR40 in fatty acid action on the beta cell line INS-1E. Biochemical and Biophysical Research Communications 335(1):97–104.
[12] Tang, C., Ahmed, K., Gille, A., Lu, S., Grone, H.-J., Tunaru, S., et al., 2015. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. Nature Medicine 21(2):173–177.
[13] Layden, B.T., Angueira, A.R., Brodsky, M., Dauri, V., Lowe Jr., W.L., 2013. Short chain fatty acids and their receptors: new metabolic targets. Translational Research 161(3):131–140.
Prentki, M., Vischer, S., Glennon, M.C., Regazzi, R., Deeney, J.T., Corkey, B.E., 1992. Malonyl-coa and long-chain acyl-coa esters as metabolic coupling factors in nutrient-induced insulin secretion. Journal of Biological Chemistry 267(9):5802–5810.

Heissig, H., Urban, K.A., Hastedt, K., Zunkler, B.J., Panten, U., 2005. Mechanism of the insulin-releasing action of alpha-ketoisocaproate and related alpha-keto acid anions. Molecular Pharmacology 60(4):1097–1105.

Thams, F., Capito, K., 1999. L-Arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. European Journal of Endocrinology 140(1):87–93.

Li, C.H., Buettger, C., Kwaghi, J., Matter, A., Daikhtin, Y., Nissim, I.B., et al., 2004. A signaling role of glutamine in insulin secretion. Journal of Biological Chemistry 279(14):13393–13401.

McClanaghan, N.H., Barnett, C.R., Gharte, F.P.M., Flatt, P.R., 1996. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. Journal of Endocrinology 151(3):349–357.

Hell, N.S., de Aquiar Pupo, A., 1979. Influence of the vagus and splanchnic nerves on insulin secretion and glycemia. Journal of the Autonomic Nervous System 1(1):93–101.

Sonnenberg, O.E., Hoffmann, R.G., Johnson, C.P., Kisselbah, A.H., 1992. Low- and high-frequency insulin secretion pulses in normal subjects and pancreas transplant recipients: role of extrinsic innervation. The Journal of Clinical Investigation 90(2):545–553.

Miller, R.E., Waid, T.H., Joyce, M.P., 1976. Direct neural inhibition of insulin secretion in response to systemic hypoglycemia. American Journal of Physiology 230(4):1090–1094.

Gilon, P., Henquin, J.C., 2001. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. Endocrine Reviews 22(5):565–604.

McIntyre, N., Holdsworth, C.D., Turner, D.S., 1965. Intestinal factors in control of insulin secretion. Journal of Clinical Endocrinology & Metabolism 25(10):1317.

Drucker, D.J., Nauck, M.A., 2006. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368(9548):1696–1705.

Drucker, D.J., Philippe, J., Mojsov, S., Chick, W.L., Habener, J.F., 1987. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proceedings of the National Academy of Sciences of the United States of America 84(10):3434–3438.

Kashima, Y., Miki, T., Shibasaki, T., Ozaki, N., Miyazaki, M., Yano, H., et al., 2001. Critical role of cAMP-GEFII–Rim2 complex in incretin-potentiated insulin secretion. The Journal of Biological Chemistry 276(49):46046–46053.

Rajan, S., Dickson, L.M., Mathew, E., Orr, C.M.O., Ellenbroek, J.H., Philipson, L.H., et al., 2015. Chronic hyperglycemia downregulates GLP-1 receptor signaling in pancreatic beta-cells via protein kinase A. Molecular Metabolism 4(4):265–276.

Shigeto, M., Ramracheya, R., Tarasov, A.I., Cha, C.Y., Chibalina, M.V., Hastoy, B., et al., 2015. GLP-1 stimulates insulin secretion by PKC-dependent TRPM4 and TRPM5 activation. The Journal of Clinical Investigation 125(12):4714–4728.

Maida, A., Hansotia, T., Longuet, C., Seino, Y., Drucker, D.J., 2009. Differential importance of glucose-dependent insulinotropic polypeptide vs glucagon-like peptide 1 receptor signaling for beta cell survival in mice. Gastroenterology 137(6):2146–2157.

Baggio, L.L., Drucker, D.J., 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132(6):2131–2157.

Secher, A., Jelsing, J., Baquero, A.F., Hecksher-Sorensen, J., Cowley, M.A., Dalbog, L.S., et al., 2014. The arcuate nucleus mediates GLP-1 receptor agonist iraglutide-dependent weight loss. The Journal of Clinical Investigation 124(10):4473–4488.

Skelly, R.H., Bollheimer, L.C., Wicksted, B.L., Corkey, B.E., Rhodes, C.J., 1998. A distinct difference in the metabolic stimulus–response coupling pathways for regulating proinsulin biosynthesis and insulin secretion that lies at the level of a requirement for fatty acyl moieties. The Biochemical Journal 331(Pt 2):553–561.

Itch, M., Mandarino, L., Gerich, J.E., 1980. Antisomatostatin gamma-globulin augments secretion of both insulin and glucagon in vitro — evidence for a physiologic role for endogenous somatostatin in the regulation of pancreatic A-cell and B-cell function. Diabetes 29(6):693–696.

Ashcroft, S.J.H., 1980. Gluocceptor mechanisms and the control of insulin release and biosynthesis. Diabetologia 18(1):5–15.

Leibowitz, G., Khaldi, M.Z., Shauer, A., Barnes, M., Oprescu, A.I., Cerasi, E., et al., 2005. Mitochondrial regulation of insulin production in rat pancreatic islets. Diabetologia 48(8):1549–1559.

Bollheimer, L.C., Skelly, R.H., Chest, M.W., McGarry, J.D., Rhodes, C.J., 1998. Chronic exposure to free fatty acid reduces pancreatic beta cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. Journal of Clinical Investigation 101(5):1094–1101.

Hakan Borg, L.A., Andersson, A., 1981. Long-term effects of glibenclamide on the insulin production, oxidative metabolism and quantitative ultrastructure of mouse pancreatic islets maintained in tissue culture at different glucose concentrations. Acta Diabetologica Latina 18(1):65–83.

Maedler, K., Carr, R.D., Bosco, D., Zueilig, R.A., Berney, T., Donath, M.Y., 2005. Sulfonylurea induced beta-cell apoptosis in cultured human islets. The Journal of Clinical Endocrinology and Metabolism 90(1):501–506.

Kahn, S.E., Haffner, S.M., Heise, M.A., Herman, W.H., Holman, R.R., Jones, N.P., et al., 2006. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. The New England Journal of Medicine 355(23):2427–2443.

Alarcon, C., Verchere, C.B., Rhodes, C.J., 2012. Translational control of glucose-induced islet amyloid polypeptide production in pancreatic islets. Endocrinology 153(5):2082–2087.

Wicksted, B., Alarcon, C., Briaud, I., Lingohr, M.K., Rhodes, C.J., 2003. Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin. Journal of Biological Chemistry 278(43):42080–42090.

Ashcroft, S.J., Bunce, J., Lowry, M., Hansen, S.E., Heredos, C.J., 1978. The effect of sugars on (pro)insulin biosynthesis. The Biochemical Journal 174(2):517–526.

Ashcroft, S.J., Crossley, J.R., 1975. The effects of glucose, N-acetylglucosamine, glyceraldehyde and other sugars on insulin release in vivo. Diabetes 14(11):279–284.

Hara, E., Saito, M., 1981. Impaired insulin secretion after oral sucrose and fructose in rats. Endocrinology 109(3):966–970.

Kyriazis, G.A., Soundarapandian, M.M., Tyberg, B., 2012. Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proceedings of the National Academy of Sciences of the United States of America 109(8):E524–E532.

Ashcroft, S.J., Weerasinghe, L.C., Bassett, J.M., Randle, P.J., 1972. The pentose cycle and insulin release in mouse pancreatic islets. The Biochemical Journal 126(3):525–532.

Jain, K., Logothetopoulos, J., 1977. Stimulation of proinsulin biosynthesis by purine-ribonucleosides and D-ribose. Endocrinology 104(4):923–927.

Parry, D.G., Taylor, K.W., 1966. The effects of sugars on incorporation of [3H] leucine into insulins. The Biochemical Journal 100(1):2C–4C.

Ashcroft, S.J., Crossley, J.R., Crossley, P.C., 1976. The effect of N-acetylglucosamines on the biosynthesis and secretion of insulin in the rat. The Biochemical Journal 154(3):701–707.
Review

[50] Ashcroft, S.J., Lowry, M., 1979. Beta-cell recognition of stereoisomers of D-glucose. Diabetologia 17(3):165—168.

[51] Toyota, T., Ando, Y., Nishimura, H., Hirata, Y., 1971. Effects of maltose on insulin secretion in perfused rat pancreas. The Tohoku Journal of Experimental Medicine 104(4):325—330.

[52] Grant, A.M., Christie, M.R., Ashcroft, S.J., 1980. Insulin release from human pancreatic islets in vitro. Diabetologia 19(2):114—117.

[53] Asanuma, N., Aizawa, T., Sato, Y., Schermanthorn, T., Komatsu, M., Sharp, G.W., et al., 1997. Two signaling pathways, from the upper glycolytic flux and from the mitochondria, converge to potentiate insulin release. Endocrinology 138(2):751—755.

[54] Malaisse, W.J., Hercehuela, A., Levy, J., Sener, A., Pipeleers, D.G., Devis, G., et al., 1975. The stimulus-secretion coupling of glucose-induced insulin release XIX. The insulinotropic effect of glyceraldehyde. Molecular and Cellular Endocrinology 4(1):1—12.

[55] Sener, A., Kawazu, S., Hutton, J.C., Boscher, A.C., Devis, G., Somers, G., et al., 1978. The stimulus-secretion coupling of glucose-induced insulin release. Effect of exogenous pyruvate on islet function. The Biochemical Journal 176(1):217—232.

[56] MacDonald, M.J., Fahien, L.A., 1988. Glyceraldehyde phosphate and methyl esters of succinic acid. Two “new” potent insulin secretagogues. Diabetes 37(7):997—999.

[57] Alarcon, C., Wicksteed, B., Prentki, M., Corkey, B.E., Rhodes, C.J., 2002. Suckinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation. Diabetes 51(8):2496—2504.

[58] Malaisse, W.J., Rasschaert, J., Villanueva-Penacarrillo, M.L., Valverde, I., 1993. Respiratory, ionic, and functional effets of suckinate esters in pancreatic islets. American Journal of Physiology 264(3 Pt 1):E428—E433.

[59] Anderson, A., 1976. Stimulation of insulin biosynthesis in isolated mouse islets by L-leucine, 2-aminobornane-2-carboxylic acid and alpha-ketosacproic acid. Biochimica et Biophysica Acta 437(2):345—353.

[60] Sener, A., Hutton, J.C., Malaisse, W.J., 1981. The stimulus-secretion coupling of amino acid-induced insulin release. Synergistic effects of 2-glutamate and 2-keto acids upon insulin secretion. Biochimica et Biophysica Acta 677(1):32—38.

[61] Basabe, J.C., Lopez, N.L., Viktora, J.K., Wolff, F.W., 1971. Insulin secretion studied in the perfused rat pancreas. I. Effect of tolbutamide, leucine and arginine; their interaction with diazoxide, and relation to glucose. Diabetes 20(7):449—456.

[62] Schatz, H., Nierle, C., Huff, E.F., 1975. (Pro-) insulin biosynthesis and release of newly synthesized (pro-) insulin from isolated rat pancreas in the presence of amino acids and sulphoynlureas. European Journal of Clinical Investigation 5(6):477—485.

[63] Jain, K., Logothetopoulos, J., 1978. Metabolic signals produced by purine ribonucleosides stimulate proinsulin biosynthesis and insulin secretion. The Biochemical Journal 170(3):461—467.

[64] Andersson, A., 1978. Opposite effects of starvation on oxidation of [14C] adenosine and adenosine-induced insulin release by isolated mouse pancreatic islets. The Biochemical Journal 176(2):619—621.

[65] Berne, C., 1975. Effect of fatty acids and ketone bodies on the biosynthesis of insulin in isolated pancreatic islets of obese hyperglycemic mice. Hormone and Metabolic Research 7(5):385—389.

[66] Malaisse, W.J., Lebrun, P., Yayli, A., Camara, J., Valverde, I., Sener, A., 1990. Ketone bodies and islet function: 45Ca handling, insulin synthesis, and release. The American Journal of Physiology 259(1 Pt 1):E117—E122.

[67] Crespin, S.R., Greenough 3rd, W.B., Steinberg, D., 1969. Stimulation of insulin secretion by infusion of free fatty acids. The Journal of Clinical Investigation 48(10):1934—1943.

[68] Pingitore, A., Chambers, E.S., Hill, T., Maldonado, I.R., Liu, B., Berwick, G., et al., 2016. The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. Diabetes, Obesity and Metabolism, http://dx.doi.org/10.1111/dom.12611.

[69] Veprik, A., Laufer, D., Weiss, S., Rubins, N., Walker, M.D., 2016. GPR41 modulates insulin secretion and gene expression in pancreatic beta-cells and modifies metabolic homeostasis in fed and fasting states. FASEB Journal 30(11):3860—3869.

[70] Priyadarshini, M., Layden, B.T., 2015. FFAR3 modulates insulin secretion and global gene expression in mouse islets. Islets 7(2):e105162.

[71] Ximenes, H.M., Hirata, A.E., Rocha, M.S., Curi, R., Carpinelli, A.R., 2007. Propionate inhibits glucose-induced insulin secretion in isolated rat pancreatic islets. Cell Biochemistry and Function 25(2):173—178.

[72] Montague, W., Taylor, K.W., 1968. Regulation of insulin secretion by short chain fatty acids. Nature 217(5131):853.

[73] Genuith, S., Lebouit, H.E., 1965. Stimulation of insulin release by corticotropin. Endocrinology 76:1093—1099.

[74] Sieradzki, J., Schatz, H., Pfeiffer, E.F., 1977. Hypophysis and function of pancreatic islets. IV. Effect of treatment with growth hormone and corticotropin on insulin secretion and biosynthesis in isolated pancreatic islets of normal rats. Acta Endocrinologica (Copenh) 88(4):813—819.

[75] Billiaudel, B., Sutter, B.C., 1982. Effect of corticosterone upon insulin biosynthesis and storage by isolated rat Langerhans islets. Diabetes & Metabolism 8(4):283—287.

[76] Billiaudel, B., Sutter, B.C.J., 1979. Direct effect of corticosterone upon insulin secretion studied by 3 different techniques. Hormone and Metabolic Research 11(10):555—560.

[77] Kawabe, T., Morgan, C.R., 1983. Multiple effects of growth hormone on insulin release from isolated pancreatic islets. Metabolism 32(7):728—731.

[78] Pierluissi, J., Pierluissi, R., Ashcroft, S.J., 1982. Effects of hypophysectomy and growth hormone on cultured islets of Langerhans of the rat. Diabetologia 22(2):134—137.

[79] Samols, E., Marri, G., Marks, V., 1965. Promotion of insulin secretion by glucagon. Lancet 2(7409):415.

[80] Kawai, K., Yokota, C., Ohashi, S., Watanabe, Y., Yamashita, K., 1995. Evidence that glucagon stimulates insulin secretion through its own receptor in rats. Diabetes 38(3):274—276.

[81] Fehmann, H.C., Habener, J.F., 1992. Insulinotropic hormone glucagon-like peptide-1(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 130(1):159—166.

[82] Mojsov, S., Weir, G.C., Habener, J.F., 1987. Insulinotropic: glucagon-like peptide 1 (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. The Journal of Clinical Investigation 79(2):616—619.

[83] Schafer, R., Schatz, H., 1979. Stimulation of (Pro-) insulin biosynthesis and release by gastric inhibitory polypeptide in isolated islets of rat pancreas. Acta Endocrinologica (Copenh) 91(3):493—500.

[84] Olsson, S.E., Anderson, A., Petersson, B., Wellersström, C., 1976. Effects of somatostatin on the biosynthesis and release of insulin from isolated pancreatic islets. Diabetes & Metabolism 2(4):199—202.

[85] Nagamatsu, S., Carroll, R.J., Grodsky, G.M., Steiner, D.F., 1990. Lack of islet amyloid polypeptide regulation of insulin biosynthesis or secretion in normal rat islets. Diabetes 39(7):871—874.

[86] Nagamatsu, S., Nishi, M., Steiner, D.F., 1992. Effects of islet amyloid polypeptide (APP) on insulin biosynthesis or secretion in rat islets and mouse beta TC3 cells. Biosynthesis of APP in mouse beta TC3 cells. Diabetes Research and Clinical Practice 15(1):49—55.

[87] O’Brien, T.D., Westermark, P., Johnson, K.H., 1990. Islet amyloid polypeptide (APP) does not inhibit glucose-stimulated insulin secretion from isolated perfused rat pancreas. Biochemical and Biophysical Research Communications 170(3):1223—1228.

[88] Rhodos, C.J., Taylor, K.W., 1984. Effect of human lymphoblastoid interferon on insulin synthesis and secretion in isolated human pancreatic islets. Diabetologia 27(6):601—603.
[98] Rhodes, C.J., Taylor, K.W., 1985. Effect of interferon and double-stranded RNA on B-cell function in mouse islets of Langerhans. The Biochemical Journal 228(1):87–94.

[99] Sandler, S., Andersson, A., Hellrengel, C., 1987. Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of isolated rat pancreatic islets. Endocrinology 121(4):1424–1431.

[100] Hansen, D.S., Nielsen, J.H., Linde, S., Spinias, G.A., Weilnder, B.S., Mandrup-Poulsen, T., et al., 1988. Effect of interleukin-1 on the biosynthesis of pro-insulin and insulin in isolated rat pancreatic islets. Biomedical Biochimica Acta 47(4–5):305–309.

[101] Porte Jr., D., Graber, A.L., Kuzuya, T., Williams, R.H., 1966. The effect of epinephrine on immunoreactive insulin levels in man. The Journal of Clinical Investigation 45(2):228–236.

[102] Spinias, G.A., Hanrahan, B.S., Kastern, W., Molvig, J., Mandrup-Poulsen, T., et al., 1987. Interleukin 1 dose-dependently affects the biosynthesis of pro-insulin in isolated rat islets of Langerhans. Diabetologia 30(7):474–480.

[103] Rhodes, C.J., Taylor, K.W., 1985. Effect of interferon and double-stranded RNA on B-cell function in mouse islets of Langerhans. The Biochemical Journal 228(1):87–94.

[104] Spinias, G.A., Mandrup-Poulsen, T., Molvig, J., Baek, L., Bentzken, K., Dinarello, C.A., et al., 1986. Low concentrations of interleukin-1 stimulate and high concentrations inhibit insulin release from isolated rat islets of Langerhans. Acta Endocrinologica (Copenh) 113(4):551–558.

[105] Lebowitz, G., Oprescu, A.I., Uckaya, G., Gross, D.J., Cerasi, E., Kaiser, N., 2003. Insulin does not mediate glucose stimulation of proinsulin biosynthesis. Diabetes 52(4):998–1003.

[106] Zawalich, W.S., Zawalich, K.C., 2002. Effects of glucose, exogenous insulin, and carbachol on c-peptide and insulin secretion from isolated perfused rat islets. The Journal of Biological Chemistry 277(29):26233–26237.

[107] Markoff, E., Beattie, G.M., Hayek, A., Lewis, U.J., 1990. Effects of prolactin and glycosylated prolactin on (pro)insulin synthesis and insulin release from cultured rat pancreatic islets. Pancreas 5(1):99–103.

[108] Nielsen, J.H., 1982. Effects of growth hormone, prolactin, and placental interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of isolated rat pancreatic islets. The Biochemical Journal 228(1):87–94.

[109] Levy, J., Malaisse, W.J., 1975. The stimulation-secretion coupling of glucose-induced insulin release-XVII. Effects of sulfonlyureas and diazoxide on insulin biosynthetic activity. Biochemical Pharmacology 24(2):235–239.

[110] Wijesekara, N., Dai, F.F., Hardy, A.B., Giglou, P.R., Bhattacharjee, A., Koshkin, V., et al., 2010. Beta cell-specific ZnT8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetes 59(8):1656–1668.

[111] Sandler, S., Andersson, A., Hellerstrom, C., 1987. Inhibitory effects of epinephrine, norepinephrine, and cholinergic agents upon insulin secretion in vitro. Endocrinology 110(2):308–312.

[112] Wijesekara, N., Dai, F.F., Hardy, A.B., Giglou, P.R., Bhattacharjee, A., Koshkin, V., et al., 2010. Beta cell-specific ZnT8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetes 59(8):1656–1668.

[113] Weiss, C.J., Taylor, K.W., 1966. Effects of diazoxide on insulin secretion in vitro. Lancet 1(7429):128–129.

[114] Zawalich, W., Brown, C., Rasmussen, H., 1983. Insulin secretion: combined effects of phorbol ester and A23187. Biochemical and Biophysical Research Communications 117(2):448–455.

[115] Uchizono, Y., Alarcon, C., Wicksteed, B.L., Marsh, B.J., Rhodes, C.J., 2007. The role of calcium/calmodulin-dependent protein kinase cascade in glucose recognition of glucose by the beta-cell. Endocrinology 93(5):1012–1018.

[116] Pipeleers, D.G., Marichal, M., Malaisse, W.J., 1993. The role of Ca2+, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiological Reviews 83(4):1248–1280.

[117] Prentki, M., Matschinsky, F.M., 1987. Ca2+, GMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiological Reviews 67(4):1185–1248.

[118] Foa, P.P., Galansino, G., Pozza, G., 1956. Insulin secretion following carbutamide injections in normal dogs. Proceedings of the Society for Experimental Biology and Medicine 93(3):539–542.

[119] Lin, B.J., Haist, R.E., 1973. Effects of some modulators of insulin secretion on insulin biosynthesis. Endocrinology 92(3):735–742.

[120] Skelly, R.H., Schupp, G.T., Ishihara, H., Oka, Y., Rhodes, C.J., 1996. Glucose-regulated transcontrolional regulation of proinsulin biosynthesis with that of the proinsulin endopeptidases PC2 and PC3 in the insulin-producing MIN6 cell line. Diabetes 45(1):37–43.

[121] Mosch, K.E., Vaughan, G.D., 1967. Insulin release after ACTH, glucagon and adenosine-3’:5’-phosphate (cyclic AMP) in the perfused isolated rat pancreas. Diabetes 16(7):449–454.

[122] Comfort, C.K., Fleischner, N., Erlichman, J., 1980. Ca2+-dependent protein phosphorylation and insulin release in intact hamster insulinoma cells. Inhibition by trifluoperazine. The Journal of Biological Chemistry 255(23):11063–11066.

[123] Hollman, P.C., Baijel, E.M., Rutherford, N.G., Hutton, J.C., 1991. Insulin secretory granule biogenesis — coordinate regulation of the biosynthesis of the majority of constituent proteins. Biochemical Journal 274:73–78.

[124] Conaway, R.H., Grifley, M.A., Whitney, J.E., 1975. Characterization of acetylcholine-induced insulin secretion in the isolated perfused dog pancreas. Proceedings of the Society for Experimental Biology and Medicine 150(2):308–312.

[125] Pielleers, D.G., Marichal, M., Malaisse, W.J., 1973. The stimulus-secretion coupling of glucose-induced insulin release. XVI. A glucose-like and calcium-independent messenger of glucose-induced insulin release. XXXIX. Long term effects of K+ deprivation upon insulin secretion and release. Endocrinology 106(3):778–785.

[126] Zawalich, W.S., Zawalich, K.C., 2002. Effects of glucose, exogenous insulin, and carbachol on C-peptide and insulin secretion from isolated perfused rat islets. The Journal of Biological Chemistry 277(29):26233–26237.

[127] Conaway, R.H., Grifley, M.A., Whitney, J.E., 1975. Characterization of acetylcholine-induced insulin secretion in the isolated perfused dog pancreas. Proceedings of the Society for Experimental Biology and Medicine 150(2):308–312.

[128] Conaway, R.H., Grifley, M.A., Whitney, J.E., 1975. Characterization of acetylcholine-induced insulin secretion in the isolated perfused dog pancreas. Proceedings of the Society for Experimental Biology and Medicine 150(2):308–312.

[129] Conaway, R.H., Grifley, M.A., Whitney, J.E., 1975. Characterization of acetylcholine-induced insulin secretion in the isolated perfused dog pancreas. Proceedings of the Society for Experimental Biology and Medicine 150(2):308–312.

[130] Conaway, R.H., Grifley, M.A., Whitney, J.E., 1975. Characterization of acetylcholine-induced insulin secretion in the isolated perfused dog pancreas. Proceedings of the Society for Experimental Biology and Medicine 150(2):308–312.
Halban, P.A., Wolleheim, C.B., 1980. Intracellular degradation of insulin stores by rat pancreatic islets in vitro — an alternative pathway for homeostasis of pancreatic insulin content. Journal of Biological Chemistry 255(13):6003–6006.

Marsh, B.J., Soden, C., Alarcon, C., Wicksteed, B.L., Yaekura, K., Costin, A.J., et al., 2007. Regulated autophagy controls hormone content in secretory-deficient pancreatic endocrine beta-cells. Molecular Endocrinology 21(9):2255–2269.

Glaumann, H., Ahlberg, J., Marzella, L., 1980. In vitro uptake of particles by lysosomes by means of microautophagy. European Journal of Cell Biology 22(1):200.

Marzella, L., Ahlberg, J., Glaumann, H., 1981. Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. Virchows Arch B-Cell Pathology Including Molecular Pathology 36(2–3):219–234.

Goginashvili, A., Zhang, Z., Erts, E., Spiegelhalter, C., Kessler, P., Mihran, M., et al., 2015. Insulin secretory granules control autophagy in pancreatic beta-cells. Science 347(6224):876–882.

Chakravarty, M.V., Booth, F.W., 2004. Eating, exercise, and “thrifty” genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases. Journal of Applied Physiology 96(1):10–30.

Neel, J.V., 1962. Diabetes mellitus — a thrifty genotype rendered detrimental by progress. American Journal of Human Genetics 14(4):353.

Nerenberg, S.T., 1953. Regranulation of beta-cells of islets of langerhans following insulin and starvation. American Journal of Clinical Pathology 23(4):340–342.

Lever, J.D., Findlay, J.A., 1964. Specific granularity in pancreatic beta-cells of starved + free-fed Guinea-pig — quantitative assessment. Journal of Anatomy 98(1):55.

Miyaura, C., Chen, L., Appel, M., Alam, T., Blaichman, J., Moosavi, M., et al., 2010. Beta-cell mass dynamics and islet cell plasticity in human type 2 diabetes. Endocrinology 151(4):1462–1472.

Maclean, N., Ogilvie, R.F., 1955. Quantitative estimation of the pancreatic islet tissue in diabetic subjects. Diabetes 4(5):367–376.

Butler, A.E., Biddinger, S.B., Kahn, C.R., 2006. From mice to men: insights into the insulin resistance syndromes. Annual Review of Physiology, 123–138.

Michael, M.D., Kulkarni, R.N., Postic, C., Prentki, M.L., Shulman, G.I., Magnuson, M.A., et al., 2000. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Molecular Cell 6(1):87–97.

Bluher, M., Michael, M.D., Peroni, O.D., Ueki, K., Carter, N., Kahn, B.B., et al., 2002. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Developmental Cell 3(1):25–38.

Cariou, B., Postic, C., Boudou, P., Burcelin, R., Kahn, C.R., Girard, J., et al., 2004. Cellular and molecular mechanisms of adipose tissue plasticity in muscle insulin receptor knockout mice. Endocrinology 145(4):1926–1932.

Meier, J.J., 2008. Beta cell mass in diabetes: a realistic therapeutic target? Diabetologia 51(5):703–713.

Saxena, R., Voight, B.F., Lyssenko, V., Baur, N.P., de Bakker, P.I.W., Chen, H., et al., 2007. Genome-wide association study identifies loci for type 2 diabetes and triglyceride levels. Science 316(5829):1341–1346.

Sladek, R., Rocheleau, G., Rung, J., Dina, C., Shen, L., Serre, D., et al., 2007. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445(7130):881–885.

Scott, L.J., Mihike, K.L., Bonnycastle, L.L., Willer, C.J., Li, Y., Duren, W.L., et al., 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316(5829):1341–1345.

Harris, M.I., 1995. Epidemiologic studies on the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM). Clinical and Investigative Medicine 18(4):231–239.

Lupi, R., Dotta, F., Marselli, L., Del Guerra, S., Masini, M., Santangelo, C., et al., 2002. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 51(5):1437–1442.

Wojcik, R., 2007. beta-cell failure in diabetes and preservation by clinical treatment. Endocrine Reviews 28(2):187–218.

Poulton, V., Robertson, R.P., 2008. Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocrine Reviews 29(3):351–366.

Logothet, J., Davidson, J.K., Halst, R.E., Best, C.H., 1965. Degranulation of beta cells and loss of pancreatic insulin after infusions of insulin antibody or glucose. Diabetes 14(8):493.

van Raalte, D.H., Verchere, C.B., 2016. Glucagon-Like Peptide-1 receptor agonists: beta-cell protection or exhaustion? Trends Endocrinol Metab 27(7):442–445.

Alarcon, C., Wicksteed, B., Rhodes, C.J., 2006. Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. Diabetologia 49(12):2920–2929.

Ogilvie, R.F., 1937. A quantitative estimation of the pancreatic islet tissue. Quarterly Journal of Medicine 6(23):287–300.

Hanley, S.C., Austin, E., Assouline-Thomas, B., Kapeluto, J., Blaichman, J., Moosavi, M., et al., 2010. Beta-cell mass dynamics and islet cell plasticity in human type 2 diabetes. Endocrinology 151(4):1462–1472.
Weir, G.C., Bonner-Weir, S., 2004. Five stages of evolving beta-cell metabolism during progression to diabetes. Diabetes 53:S16–S21.

Alarcon, C., Boland, B.B., Uchizono, Y., Moore, P.C., Peterson, B., Rajan, S., et al., 2016. Pancreatic beta-cell adaptive plasticity in obesity increases insulin production but adversely affects secretory function. Diabetes 65(2):438–450.

Rutkowski, J.M., Stern, J.H., Scherer, P.E., 2015. The cell biology of fat expansion. The Journal of Cell Biology 208(5):501–512.

Kahn, S.E., Hum, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444(7121):840–846.

Shoelson, S.E., Lee, J., Goldfine, A.B., 2006. Inflammation and insulin resistance. The Journal of Clinical Investigation 116(7):1793–1801.

Agrawal, N.K., Kant, S., 2014. Targeting inflammation in diabetes: newer therapeutic options. World Journal of Diabetes 5(5):697–710.

Cao, P., Marek, P., Noor, H., Patsalo, V., Tu, L.-H., Wang, H., et al., 2013. Islet amyloid: from fundamental biophysics to mechanisms of cytotoxicity. Fems Letters 587(8):1106–1118.

Hull, R.L., Westermark, G.T., Westermark, P., Kahn, S.E., 2006. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. Journal of Clinical Endocrinology & Metabolism 89(8):3629–3643.

Clark, A., Nilsson, M.R., 2004. Islet amyloid: a complication of islet dysfunction or an aetiological factor in Type 2 diabetes? Diabetologia 47(2):157–169.

Lee, K.H., Tirasphoph, W., Okada, T., Yoshida, H., Mori, K., Kaufman, R.J., 2002. Cooperation of IRE1 alpha, ATF6, and XBP1 in the unfolded protein response in the endoplasmic reticulum (ER). Faseb Journal 16(4):A558–A.

Back, S.H., Kaufman, R.J., 2012. Endoplasmic reticulum stress and type 2 diabetes. Annual Review of Biochemistry 81:767–793.

Izumi, T., Yokota-Hashimoto, H., Zhao, S., Wang, J., Halban, P.A., Takeuchi, T., 2003. Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. Diabetes 52(2):409–416.

Sun, J., Cui, J., He, Q., Chen, Z., Arvan, P., Liu, M., 2015. Proinsulin misfolding and endoplasmic reticulum stress during the development and progression of diabetes. Molecular Aspects of Medicine 42:105–118.

Svítz, W.J., Yorek, M.A., 2010. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxidants & Redox Signaling 12(4):537–577.

Sigfrid, L.A., Cunningham, J.M., Beeharry, N., Borch, L.A.H., Hernandez, A.L.R., Carlsson, C., et al., 2004. Antioxidant enzyme activity and mRNA expression in the islets of Langerhans from the BB/S rat model of type 1 diabetes and an insulin-producing cell line. Journal of Molecular Medicine-JMM 82(9):325–335.

Leclercq, C., Tourel-Cuzin, C., Magnan, C., Karaca, M., Castel, J., Carneiro, L., et al., 2009. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes 58(3):673–681.

Affourtit, C., Brand, M.D., 2008. Activity of uncoupling protein-2 in pancreatic beta cells. Biochimica Et Biophysica Acta-Bioenergetics 1777:S76–S77.

Affourtit, C., Jasbro, M., Brand, M.D., 2011. Uncoupling protein-2 attenuates glucose-stimulated insulin secretion in INS-1E insulinoma cells by lowering mitochondrial reactive oxygen species. Free Radical Biology and Medicine 50(5):609–616.

Bashan, N., Kovash, J., Kachko, I., Ovadia, H., Rudich, A., 2009. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiological Reviews 89(1):27–71.

Kahn, S.E., Prigeon, R.L., McCulloch, D.K., Boyko, E.J., Bergman, R.N., Schwartz, M.W., et al., 1993. Quantification of the relationship between insulin sensitivity and beta-cell function in human-subjects — evidence for a hyperbolic function. Diabetes 42(11):1663–1672.

Peyot, M.-L., Pepin, E., Lamartangne, J., Latour, M.G., Zarrouki, B., Lussier, R., et al., 2010. Beta-cell failure in diet-induced obese mice stratified according to body weight gain: secretory dysfunction and altered islet lipid metabolism without steatosis or reduced beta-cell mass. Diabetes 59(9):2178–2187.

Pfeifer, M.A., Halter, J.B., Porte, D., 1981. Insulin-secretion in diabetes-mellitus. American Journal of Medicine 70(3):579–588.

Ward, W.K., Bolgiano, D.C., McKnight, B., Halter, J.B., Porte, D., 1984. Diminished B-cell secretory capacity in patients with noninsulin-dependent diabetes-mellitus. Journal of Clinical Investigation 74(4):1316–1328.

Rask, E., Olsson, T., Soderberg, S., Johnson, O., Seck, J., Holst, J.J., et al., 2001. Improved incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. Diabetes Care 24(9):1640–1645.

Ward, W.K., Lacava, E.C., Paquette, T.L., Beard, J.C., Wallum, B.J., Porte, D., 1967. Disproportionate elevation of immunoreactive proinsulin in type-2 (non-insulin-dependent) diabetes-mellitus and in experimental insulin resistance. Diabetologia 30(9):698–702.

Polonsky, K.S., Given, B.D., Hirsch, L.J., Tillil, H., Shapiro, E.T., Beebe, C., et al., 1988. Abnormal patterns of insulin-secretion in non-insulin-dependent diabetes-mellitus. New England Journal of Medicine 318(19):1231–1239.

Kanda, Y., Hashimamoto, M., Shimoda, M., Hamamoto, S., Tawakomato, K., Kimura, T., et al., 2015. Dietary restriction preserves the mass and function of pancreatic beta cells via cell kinetic regulation and suppression of oxidative/ER stress in diabetic mice. The Journal of Nutritional Biochemistry 26(3):219–226.

Sathyanathan, M., Shah, M., Edens, K.L., Grothe, K.B., Piccinini, F., Farrugia, L.P., et al., 2015. Six and 12 weeks of caloric restriction increases beta cell function and lowers fasting and postprandial glucose concentrations in people with type 2 diabetes. The Journal of Nutrition 145(9):2046–2051; Knowler, W.C., Barrett-Connor, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Walker, E.A., et al., 2002. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. The New England Journal of Medicine 346(6):393–403.

Johnson, J.B., Summer, W., Cutler, R.G., Martin, B., Hyun, D.H., Dixit, V.D., et al., 2007. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radical Biology and Medicine 42(5):665–674.

Greenwood, R.H., Mahler, R.F., Hales, C.N., 1976. Improvement in insulin secretion in diabetes after dioxazone. Lancet 1(7957):444–447.

Song, S.H., Rhodes, C.J., Veldhuis, J.D., Butler, P.C., 2003. Dioxazone attenuates glucose-induced defects in first-phase insulin release and pulsatile insulin secretion in human islets. Endocrinology 144(8):3399–3405.

Glaser, B., Leibovich, G., Nesher, R., Hartling, S., Binder, C., Cerasi, E., 1988. Improved beta-cell function after intensive insulin treatment in severe non-insulin-dependent diabetes. Acta Endocrinologica (Copenh) 118(3):365–373.

DeFronzo, R.A., 2009. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773–795.

DeFronzo, R.A., Eldor, R., Abdul-Ghani, M., 2013. Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. Diabetes Care 36(Suppl 2):S127–S138.

Zinnman, B., Gerich, J., Buse, J.B., Lewin, A., Schwartz, S., Raskin, P., et al., 2009. Efficacy and safety of the human glucagon-like peptide-1 analogiraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met+TZD). Diabetes Care 32(7):1224–1230.

Busc, M.C., Diamant, M., Corner, A., Eliasson, B., Malloy, J.L., Shaginian, R.M., et al., 2009. One-year treatment with exenatide improves beta-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial. Diabetes Care 32(3):762–768.

Busc, M.C., Corner, A., Eliasson, B., Heine, R.J., Shaginian, R.M., Taekinen, M.R., et al., 2011. Effects of exenatide on measures of beta-cell function after 3 years in metformin-treated patients with type 2 diabetes. Diabetes Care 34(9):2041–2047.
Van Raalte, D.H., van Genugten, R.E., Eliasson, B., Moller-Goede, D.L., Mari, A., Tura, A., et al., 2014. The effect of alogliptin and pioglitazone combination therapy on various aspects of beta-cell function in patients with recent-onset type 2 diabetes. European Journal of Endocrinology 170(4): 565–574.

Yang, T., Lu, M., Ma, L., Zhou, Y., Cui, Y., 2015. Efficacy and tolerability of canagliflozin as add-on to metformin in the treatment of type 2 diabetes mellitus: a meta-analysis. European Journal of Clinical Pharmacology 71(11): 1325–1332.

Ohn, J.H., Kwak, S.H., Cho, Y.M., Lim, S., Jang, H.C., Park, K.S., et al., 2016. 10-year trajectory of beta-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. Lancet Diabetes Endocrinol 4(1):27–34.

Marathe, P.H., Gao, H.X., Close, K.L., 2017. American diabetes association standards of medical care in diabetes 2017. Journal of Diabetes 9(4):320–324.

Zhang, X., Gregg, E.W., Williamson, D.F., Barker, L.E., Thomas, W., Bullard, K.M., et al., 2010. A1C level and future risk of diabetes: a systematic review. Diabetes Care 33(7):1665–1673.