Review

MOLECULAR METABOLISM

The dynamic plasticity of insulin production in β-cells

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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 overnutrition, 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 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 $FADH_2$, 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 consequentially 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 Ca^{2+}channels (VDCCs) and rapid influx of Ca^{2+} into the cell. The rise in intracellular cytosolic Ca^{2+} 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.

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Abbreviations: ATF6, Activating Transcription Factor 6; CHOP, CCAAT/Enhancer-Binding Homologous Protein; EPAC, Exchange Factor Directly Activated by cAMP; EROβ1, ER-resident oxidoreductase β1; GIP, Gastric Inhibitory Polypeptide; GLP-1, Glucagon-like Peptide 1; GLUT2, Glucose Transporter 2; GSIS, Glucose Stimulated Insulin Secretion; IREα, Inositol Requiring Enzyme α; mTORC1, Mammalian Target of Rapamycin 1; NEFA, Non-esterified Fatty Acid; nH, Hill coefficient; PERK, Protein Kinase RNA-like Endoplasmic Reticulum Kinase; PKA, Protein Kinase A; PKC, Protein Kinase C; PLC, Phospholipase C; ROS, Reactive Oxygen Species; SNAP-25, Soluble NSF Attachment Protein 25; SNARE, Soluble NSF Attachment Protein Receptor; STZ, Streptozotocin; T2DM, Type 2 Diabetes Mellitus; TRP, Transient Receptor Potential; VAMP-2, Vehicle Associated Membrane Protein 2; VDCC, Voltage Dependent Calcium Channel

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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 antidiabetic 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 cAMP-dependent; both PKA and, to a lesser extent, EPAC activation contribute to the dose-dependent increase in glucose-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^{2+}-mediated depolarization and insulin secretion [28]. 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) [32]. 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 sulfonylurea 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 sulfonylureas led 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 sulfonylureas 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 glucose independent of β-cell depolarization and Ca^{2+} [126,127]. Longer term (8 h+) glucose administration can stabilize preproinsulin mRNA through an UUGAA 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-

Table 1 — 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]
Mannoheptulose	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	Conflicting reports in literature	[43,48]
N-acetylglucosamine	Slight stimulus	Stimulates		[43,49]
Other glucose stereoisomers	No effect	No effect	Includes allose, altrose, gulose, idose, talose	[50]
Other sugars	No effect	No effect	Includes sucrose, sorbitol, 2-deoxyglucose, 3-O-methylglucose, maltose, lactose	[34,51]
Dihydroxyacetone	Slight stimulus	Stimulates		[42,52]
Glyceraldehyde	Slight stimulus	Stimulates	Potentiates glucose stimulation	[43,47,53,54]
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-Ketoisocaproate	Stimulates	Stimulates	Potentiates glucose stimulation	[52,53,59]
Leucine	Stimulates	Stimulates	Only in the presence of glutamine	[52,59]
Glutamine	No effect	No effect		[32,60]
Arginine	No effect	Stimulates	Possibly stimulates insulin secretion by depolarization	[16,61,62]
Inosine	Slight stimulus	Slight stimulus		[42,47,63]
Guanosine	Slight stimulus	Slight stimulus		[47,63]
Adenosine	Slight stimulus	Slight stimulus		[63,64]
Ketone bodies	Slight stimulus	Stimulates		[52,65,66]
Long Chain Fatty Acids	No Effect	Stimulates		[65,67]
Short Chain Fatty Acids	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 *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	Slight inhibition	No effect	Inhibits proinsulin biosynthesis at very high concentrations	[88,89]
Inteleukin-1β	+/−	+/−	Concentration dependent	[90–93]
Insulin	No effect	No effect	No autocrine *direct* effect	[94,95]
Prolactin	No effect	Stimulates	Increases overall islet insulin synthesis via proliferative effect	[96–98]
Neurotransmitters				
Acetylcholine	No effect	Stimulates	Potentiates glucose stimulation	[99,100]
Epinephrine	Inhibits	Inhibits	Inhibits glucose stimulation	[101–103]
Ions				
Ca^{2+}	Inhibits	Stimulates	Required for glucose stimulated insulin secretion. Inhibits general protein synthesis	[104–107]
K+	Stimulates	Stimulates	Stimulates insulin secretion by depolarization	[104,108,109]
Mg^{2+}	Required	No effect	Required for proinsulin biosynthesis	[104,107]
Zn^{2+}	No effect	Inhibits	Involved in insulin crystal formation for storage in mature β-granules	[110–112]
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	CaM Kinase-II inhibition affects insulin secretion	[121]

terminal signal sequence enabling the newly formed preproinsulin 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 preproinsulin is rapidly cleaved, likely co-translationally, 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 granules form. Here, proinsulin to insulin and C-peptide processing begins by the action of two Ca^{2+}-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 Ca^{2+} into an immature β-granule initiate proinsulin processing and retain insulin accumulation within the organelle where it is stored

[135]. 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 [136]. 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 constituents. 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, RalA, 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].

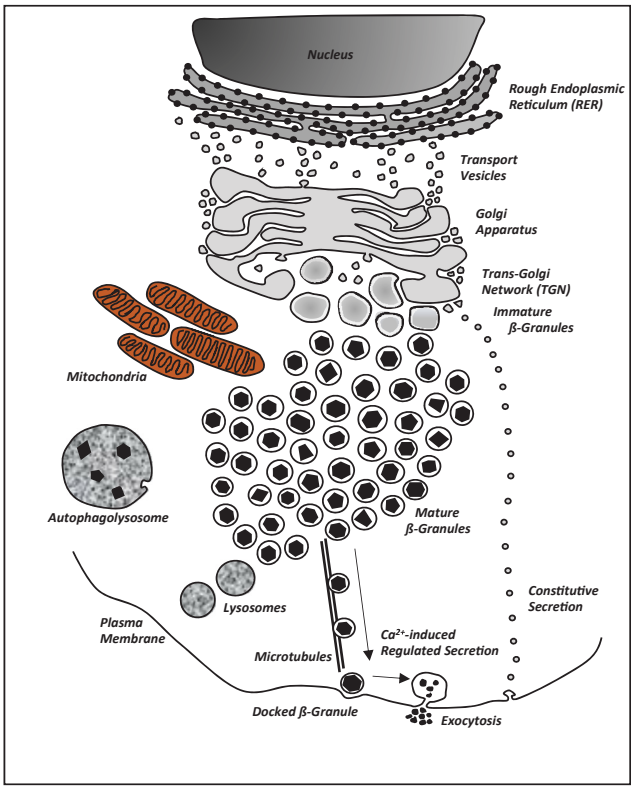
Not all insulin granules undergo exocytosis, and if retained for ~5 days they are targeted for internal degradation by autophagy [122]. Normally, this system plays a janitorial role in maintaining optimal *functional* insulin secretory stores in the β-cell [167]. It has been presumed that this turnover 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].

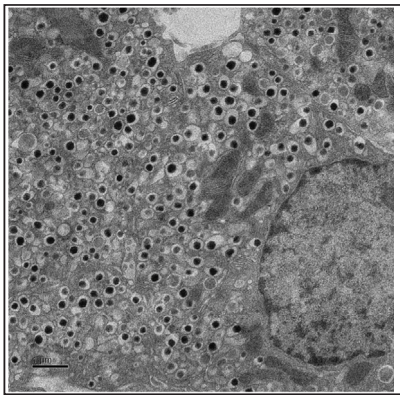
As depicted in Figure 1, under normal circumstances, a large and rather stable number of mature insulin granules characteristically mark normal β-cell biology, and this population is maintained via equilibrium between secretion, biosynthesis, and degradation. However, if this balance is disrupted, such 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

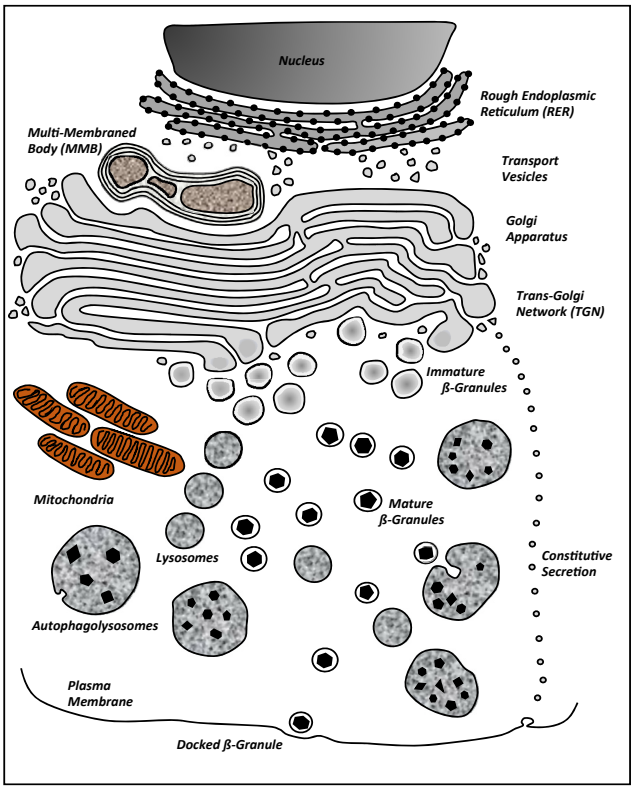
The modern human genome was likely selected during the late Paleolithic era, when humans sustained themselves primarily as hunter-gatherers [172]. Early humans thrived in an environment characterized by intermittent periods of fasting and feasting. The selection of regulatory metabolic genes that protected 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

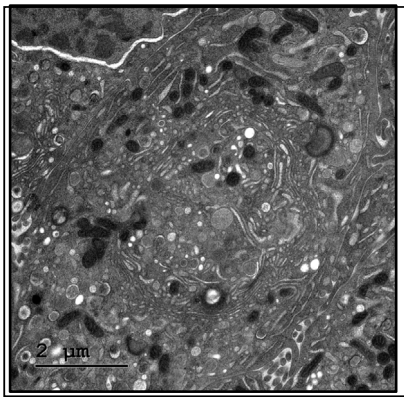




Representative β-cell from lean BL/6 mouse containing abundant mature secretory granules and very few proinsulin-containing immature granules. In this state, postprandial demand can be quickly managed by robust biphasic insulin secretion (2,500X).

Figure 1: A graphic representation and a transmission electron micrograph of a 12-week old lean C57/B6 β-cell demonstrating an abundance of mature insulin secretory granules and few immature proinsulin-containing granules. Magnification, 2500×.





Representative β-cell from a 72-hour fasted Wistar rat containing a paucity of mature secretory granules and a marked increase in immature granules. Here, the absence of a reserve pool of readily releasable insulin protects against hypoglycemia. Of note, an abundance of Golgi allows the β-cell to rapidly replenish insulin stores upon refeeding (2,500X).

Figure 2: A graphic representation and a transmission electron micrograph of a 3-day fasted 12-week old Wistar rat β-cell showing degranulation of mature insulin secretory granules, a noticeable increase in immature granules, the presence of autophagolysosomes, and an expansion of the Golgi apparatus. Magnification, 2500×.

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 islet 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, amyloidal 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 sufonylureas, 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 prediabetes [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 apparatus, 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 that lean $KS^{+/+}$ 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 of 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

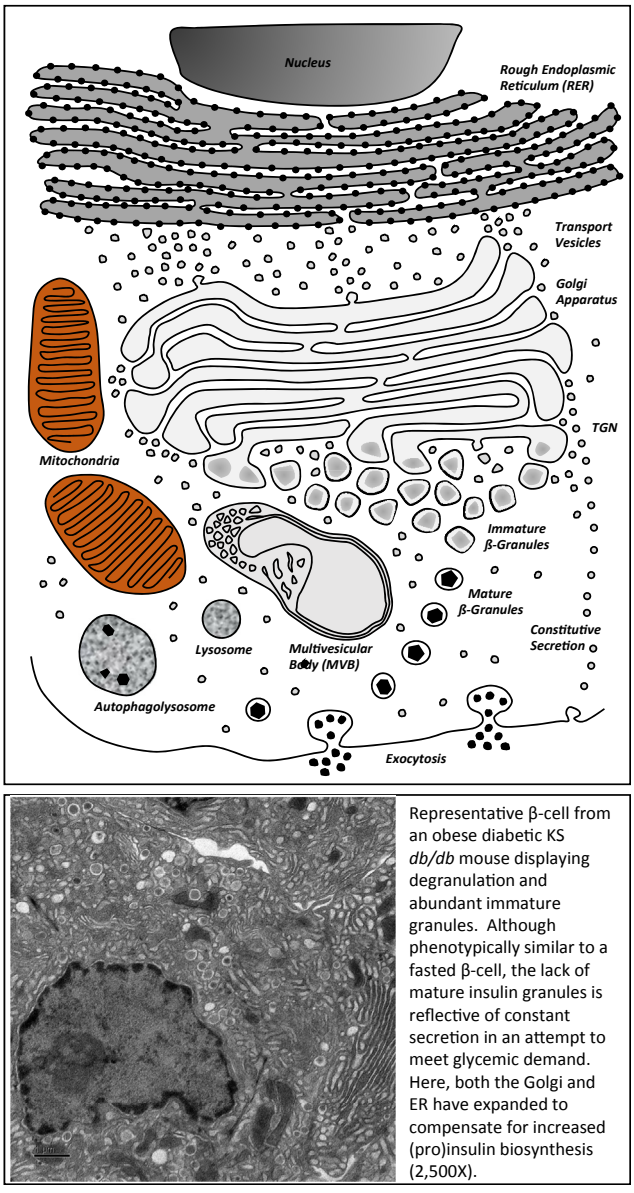


Figure 3: A graphic representation and a transmission electron micrograph of a 12-week old obese diabetic KS *db/db* showing marked degranulation of mature insulin secretory granules, abundant immature granules, and an expansion of both the RER and Golgi apparatus. Magnification, 2500×.

effect of increased internal and external fatty acids on the β-cell is termed lipotoxicity. While fatty acids can potentiate insulin secretion, the absence of a complementary increase in proinsulin biosynthesis can deplete β-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-α, IL-6, and IFN-γ, 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 β-cells that influence insulin secretion and survival [213].

Factors intrinsic to the β-cell may play a more prominent role. One notable factor in humans, islet amyloid polypeptide (amylin, or IAPP) is co-secreted with insulin and usually exists in a soluble form [214]. Like most other β-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 [209], IAPP can fold into cytotoxic aggregates, which have been implicated in β-cell apoptosis in primates [180]. IAPP aggregates form prior to the onset of frank T2DM during fasting hyperglycemia, implicating the onset of β-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].

A frequently attributed factor in secretory dysfunction and apoptosis of β-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, IRE1α, 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, β-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 β-cell translational load, it is not unreasonable to assume that the β-cell routinely manages a substantial amount of protein misfolding [220]. However, this notion has been recently challenged [201]. In β-cells of obese diabetic KS$^{db/db}$ 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 β-cells in the pathogenesis of common obese T2DM, ER-stress is unlikely to play a significant role to β-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 H_2O. However, a small percentage of the O_2 is converted to free radicals, such as superoxide anion ($\bullet O_2^-$), which are usually rapidly neutralized by a combination of cellular antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and catalase [221]. Pancreatic β-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, β-cells are deficient in H_2O_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 $\bullet O_2^-$ and regulates the rate of respiration, mitochondrial membrane potential, ATP production, and insulin release [224]. Thus, it can protect β-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

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 [226]. 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, underlying 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 $KS^{db/db}$ 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 *db/+* 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 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 Trail (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 liraglutide 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 liraglutide 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 exenatide 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-IV inhibition + TZD 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.

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