

Failure of beta-cell adaptation in type 2 diabetes: Lessons from animal models

Nurit Kaiser, Gil Leibowitz

Endocrinology and Metabolism Service, Department of Medicine, Hadassah - Hebrew University Medical Center, Jerusalem 91120, Israel

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Nutrient sensing and regulation of insulin secretion, proinsulin biosynthesis and beta-cell mass
 - 3.1. Nutrient stimulation of insulin secretion
 - 3.2. Nutrient stimulation of proinsulin biosynthesis
 - 3.3. Nutrient regulation of beta-cell mass
4. Determinants of efficient beta-cell compensation
 - 4.1. Intrinsic genetic factors and metabolic imprinting
 - 4.2. Metabolic cues for beta-cell compensation
 - 4.3. Signaling pathways involved in beta-cell compensation
5. Beta-cell failure in type 2 diabetes
 - 5.1. Beta-cell secretory dysfunction
 - 5.2. Deficient proinsulin biosynthesis
 - 5.3. Decreased beta-cell mass
6. Factors driving beta-cell failure
 - 6.1. Glucotoxicity
 - 6.2. Glucolipotoxicity
 - 6.3. Proinflammatory cytokines and adipokines
 - 6.4. Islet amyloid polypeptide
7. Deranged cellular pathways involved in beta-cell failure in type 2 diabetes
 - 7.1. Inflammatory stress
 - 7.2. Oxidative stress
 - 7.3. Endoplasmic reticulum stress
8. Concluding comments and clinical implications
9. References

1. ABSTRACT

The pancreatic beta-cell adapts to increased nutrient availability and insulin resistance by increasing its function and mass. These processes are orchestrated by signals derived from nutrient metabolism, hormones and cytokines. Their end-result is the regulation of insulin secretion and biosynthesis, and beta-cell proliferation and apoptosis. This review focuses on the mechanisms involved in beta-cell nutrient sensing and adaptation and the potential causes of beta-cell dysfunction and death in type 2 diabetes mellitus. Understanding the mechanisms that regulate adequate beta-cell adaptation and the natural history of beta-cell failure is of utmost importance for the development of novel disease modifying treatments.

2. INTRODUCTION

The growth and function of the endocrine pancreas are programmed to adapt to physiological challenges with the aim of maintaining glucose homeostasis. The pancreatic beta-cell adapts to nutrient overload and insulin resistance by augmenting insulin secretion and biosynthesis and expansion of beta-cell mass (1, 2). These processes are orchestrated by metabolic cues generated during nutrient metabolism, as well as by secreted hormones and cytokines that regulate beta-cell function, proliferation and apoptosis.

The processes that determine beta-cell adaptation to nutrient overload and to obesity-associated insulin

Failure of beta-cell adaptation in type 2 diabetes

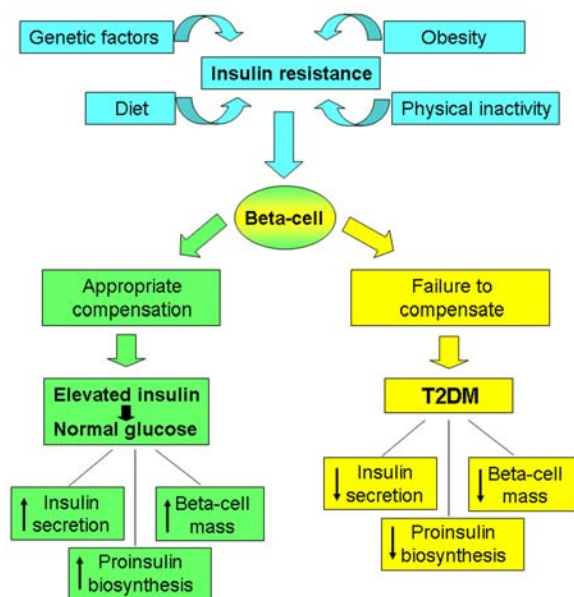


Figure 1. Factors involved in the pathophysiology of type 2 diabetes.

resistance involve multiple genes (3-5). Moreover, various environmental factors *in utero* and during adult life are also involved (reviewed in (6)). The interactions between these factors are complex, making it difficult to decipher the critical pathways that mediate the beta-cell response in states of insulin resistance and in type 2 diabetes mellitus (T2DM). Nevertheless, studies in animal models of insulin resistance and in islets of T2DM patients unraveled important pathways in nutrient sensing and their involvement in beta-cell adaptation.

There is evidence that beta-cell failure is the mainstay of the development and progression of T2DM (7). In part, this results from decreased functional beta-cell mass due to metabolic stress induced by hyperglycemia and hyperlipidemia (reviewed in (6, 8)). Among the mechanisms that may explain beta-cell failure and death in T2DM are inflammatory, oxidative and endoplasmic reticulum stress.

This review describes pathways involved in beta-cell adaptation to increased secretory demand and outlines potential mechanisms that could lead to beta-cell failure and T2DM (Figure 1)

3. NUTRIENT SENSING AND REGULATION OF INSULIN SECRETION, PROINSULIN BIOSYNTHESIS AND BETA-CELL MASS

3.1. Nutrient stimulation of insulin secretion

The primary function of the pancreatic beta-cell is to secrete insulin in response to nutrients, most notably glucose. It is well established that glucose must be metabolized in the beta-cells to provide signals for insulin secretion (9). Glucose is metabolized to pyruvate *via* glycolysis, which enters the mitochondria and undergoes

oxidative phosphorylation in the tricarboxylic acid (TCA) cycle. Pyruvate carbon metabolism promotes electron transfer from the TCA cycle to the respiratory chain by NADPH and FADH₂ leading to generation of ATP, which is exported to the cytosol. The increase in the cytosolic ATP/ADP ratio causes depolarization of the plasma membrane by closure of ATP-sensitive K⁺ channels with subsequent opening of voltage-sensitive calcium channels (10, 11). An increase in cytosolic calcium is the main stimulus for exocytosis of the insulin granules (12, 13). Glucose oxidation in the TCA cycle leads to generation of additional intermediates that may serve as signals for insulin secretion. As an example, it has been recently suggested that reactive oxygen species (ROS) generated by the electron transport chain function as important signals that couple glucose stimulation to insulin secretion (14). Efflux of mitochondrial intermediates to the cytosol and the refilling of TCA cycle intermediates (cataplerosis and anaplerosis) may also serve as important messengers for glucose-stimulated insulin secretion and are thus important nutrient sensors in the pancreatic beta-cells (15-19). Moreover, signals derived from anaplerotic shuttles, for example NADPH, may interact with other metabolic pathways, such as nitric oxide (NO) synthase and derivatives of lipid metabolism to regulate insulin secretion (20-22).

Glucose-stimulated insulin secretion is amplified by signals derived from lipid metabolism (21, 23, 24), parasympathetic activity, in part through activation of protein kinase C (25) and incretins, such as GLP-1, which augment cyclic AMP activity (26). In quantitative terms these amplifiers have a major contribution to insulin secretion in response to nutrients (27). Free fatty acids (FFAs) augment insulin secretion by binding to the membrane receptor GPR40 leading to increased intracellular calcium (28, 29). In addition, FFAs can be oxidized in the TCA cycle or directed to the synthesis of long-chain acyl CoAs. The metabolic fate of FFAs is determined by the anaplerotic product malonyl CoA, which inhibits FFA oxidation and favors the synthesis of long-chain acyl-CoAs (21, 30). Prentki and Corkey suggested that malonyl CoA serves as an important signal for glucose-stimulated insulin secretion by generating fatty acid esterification products such as diacylglycerol (DAG) (31). Malonyl CoA is generated from acetyl CoA by the enzyme acetyl CoA carboxylase (ACC). The activity of ACC is regulated by AMP kinase (AMPK), which phosphorylates the enzyme and inhibits its activity. AMPK is activated by ATP depletion and increase in cellular AMP (32-34); its activation inhibits ATP-consuming pathways, such as partitioning of FFAs to acyl CoAs, and promotes ATP production by stimulating FFA oxidation (30). Therefore, AMPK is a key nutrient sensor regulating the cellular response to changes in energy balance. AMPK converges signals from nutrients and adipokines, such as leptin and adiponectin to regulate metabolic activity in muscle, liver and the brain (35). In islets, AMPK activation was shown to inhibit insulin secretion (36, 37). However, it was recently reported that inhibition of AMPK by glucose and amino acids does not play an important role in nutrient stimulation of insulin secretion (38); therefore, the role of

Failure of beta-cell adaptation in type 2 diabetes

AMPK in the regulation of insulin secretion is controversial.

3.2. Nutrient stimulation of proinsulin biosynthesis

Nutrient stimulation of insulin secretion is accompanied by a robust increase in proinsulin biosynthesis to replenish the beta-cell insulin stores and allow sustained insulin release under conditions of increased demand (39). In the short-term, insulin production in pancreatic beta-cells is predominantly regulated through glucose control of proinsulin mRNA translation (40, 41). It was recently shown that a short element within the 5'-untranslated region of proinsulin mRNA confers glucose regulation of proinsulin translation (42). This *cis* element specifically binds a trans-acting factor expressed in islets and remains to be identified. Notably, a new insulin splice-variant differing from the wild-type proinsulin mRNA in the 5'-untranslated region was identified in human and mouse beta-cells (43). The variant confers increased translational efficiency and is highly expressed in animal models of insulin resistance and diabetes (44). This raises the possibility that an alternative proinsulin mRNA splicing represents a mechanism for beta-cell compensation in T2DM.

The metabolic regulation of proinsulin biosynthesis is not completely understood. We have previously shown that mitochondrial ATP production is the most important factor regulating glucose-stimulated proinsulin biosynthesis (45). However, parts of the metabolic signals that mediate glucose stimulation of insulin secretion and proinsulin mRNA translation are dissimilar (20, 46, 47). For example, calcium and NADPH that are important mediators of glucose stimulated insulin secretion do not affect proinsulin biosynthesis (20, 47). Moreover, FFAs which amplify glucose-stimulated insulin secretion inhibit proinsulin biosynthesis (46). This may suggest that altered metabolic activities during chronic hyperglycemia and hyperlipidemia may differentially affect insulin secretion and proinsulin biosynthesis leading to depletion of the islet insulin content. In addition to increasing proinsulin mRNA translation, glucose stimulates proinsulin gene transcription and stabilizes the formed mRNA (48). The complex regulation of proinsulin gene transcription involves the transcription factors PDX-1, MafA and beta2/NeuroD which bind to conserved elements in the proinsulin gene upstream region (49). We and others have shown that the stimulation of proinsulin gene transcription by glucose is not required for the increase of proinsulin biosynthesis in response to acute glucose stimulation; however, it is essential for the long-term adaptation to chronic hyperglycemia (40, 41). In addition to glucose, the incretin GLP-1 also stimulates insulin gene transcription (26), whereas FFAs seem to inhibit proinsulin gene transcription by impairing PDX-1 nuclear localization and MafA expression (50, 51).

3.3. Nutrient regulation of beta-cell mass

Nutrients, hormones (e.g. insulin, GLP-1) and growth factors (e.g. IGF-1) regulate beta-cell proliferation and apoptosis, determining the beta-cell mass in the adult pancreas (2, 52). The source of "new" beta-cells in adult

life is a matter of debate. Beta-cells can be derived from the pancreatic ducts, a process called neogenesis, or from proliferation of existing beta-cells (53, 54). It has been claimed that the major source of new beta-cells is the proliferation of existing beta-cells (55-57). However, a recent elegant study using an experimental model of pancreatic duct ligation convincingly showed that the adult mouse pancreas contains endogenous endocrine islet-cell progenitors that are able to differentiate into beta-cells *in vivo* and *in vitro* (58).

The effect of nutrients, hormones and growth factors on beta-cell mass is mediated by the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway (59). Stimulation of this pathway leads to tyrosine phosphorylation of IRS2, which is the most abundant and important IRS isoform in pancreatic beta-cells (60, 61). In islets, IRS2 expression is regulated at the level of transcription by glucose and GLP-1 (62, 63) and its turnover is very rapid (62). Therefore, modulation of signals regulating its synthesis and degradation may have a strong impact on its level of expression.

IRS2 activates Akt, which plays a central role in the regulation of beta-cell differentiation, size, replication, and survival (52). Akt inhibits Foxo1 and GSK3 activities, resulting in increased PDX-1 expression and activity, thus promoting expansion of the functional beta-cell mass (64, 65). In addition, Akt inhibits pro-apoptotic genes, such as Bad and procaspase 9, and activates cell cycle regulators, e.g. cyclin D1 and the serine/threonine kinase mTOR (52). The latter integrates signals from nutrients (mainly glucose and amino acids), growth factors, hormones and cellular energy level (ATP) to regulate protein translation, and cell growth, proliferation and survival (66).

4. DETERMINANTS OF EFFICIENT BETA-CELL COMPENSATION

Nutrient overload, increased insulin resistance, and/or hyperglycemia are expected to turn on compensatory mechanisms to increase beta-cell performance. Efficient beta-cell adaptation to increased demand requires augmentation of insulin secretion, proinsulin biosynthesis and beta-cell mass. Expansion of beta-cell mass is expected to affect the maximal pancreatic capacity to secrete insulin; thus, the compensatory processes of beta-cell mass and function (insulin secretion and proinsulin biosynthesis) are tightly linked.

In rodents, insulin resistance induced by a high-fat diet or during pregnancy can lead to a 2-3-fold compensatory increase of beta-cell mass; however, in humans there is only 50% increase of beta-cell mass in obese compared to lean subjects, suggesting that the plasticity of beta-cell mass in man is lower than in rodents (67). Moreover, partial pancreatectomy in man impairs beta-cell function and results in elevated blood glucose (68, 69), emphasizing the impact of changes in beta-cell mass on insulin secretion (70). Therefore, a relatively small increase of beta-cell mass may be important for efficient beta-cell functional compensation.

4.1. Intrinsic genetic factors and metabolic imprinting

The ability of the beta-cell to adapt to increased demand may be imprinted early in life by intrinsic genetic factors and by the *in utero* metabolic environment. Large-scale genome-wide association studies identified novel susceptibility genes for T2DM (71). Some of these genetic polymorphisms are associated with increased insulin resistance, whereas others are related to impaired beta-cell function. The most important T2DM susceptibility gene identified so far is transcription factor-7-like 2 (TCF7L2), which reproducibly increases the risk for T2DM in different populations (4, 72, 73). Interestingly, impaired insulin secretion and incretin effects characterize the TCF7L2 polymorphism associated with T2DM (74). Thus, inherent genetic defects in beta-cell function might limit beta-cell compensation in adult life, increasing the risk for T2DM.

Human and rodent studies showed that a low birth weight, intrauterine growth retardation (IUGR), maternal hyperglycemia and high fat consumption are associated with increased risk for T2DM in adult life (75). Islets of IUGR mice exhibit basal hyperinsulinemia with impaired glucose-stimulated insulin secretion (76). The mechanisms by which the metabolic environment *in utero* affects beta-cell function and glucose homeostasis of the offspring during adult life are not clear. It was suggested that beta-cell dysfunction in IUGR is due to increased ROS production and accumulation of mitochondrial DNA mutations (77).

Taken together, these studies suggest that beta-cell function and possibly mass are programmed by genetic and environmental factors in pregnancy, determining the efficiency of the beta-cell adaptive response to increased demand during adulthood.

4.2. Metabolic cues for beta-cell compensation

Little is known about the mechanisms underlying beta-cell compensation in man due to the limited availability of tissue from pre-diabetics or early-stage T2DM. Therefore, the molecular basis of beta-cell adaptation to insulin resistance and hyperglycemia has been studied mainly in animal models. *Psammomys obesus* (*P. obesus*) is a good model to address questions related to the mechanisms of beta-cell adaptation and failure under conditions of nutrient overload. *P. obesus* is a desert gerbil with innate insulin resistance that develops hyperglycemia when fed a high-energy (HE) diet under conditions of reduced physical activity (39). In the initial period on HE diet, hyperglycemia is accompanied by hyperinsulinemia at the expense of a marked decrease in pancreatic insulin stores. The glucose-insulin concentration-response curve, evaluated in batch-incubated islets, revealed a leftward-shift in prediabetic *P. obesus* compared to insulin-sensitive rats, suggesting that increased glucose sensitivity is a physiological adaptive mechanism to insulin resistance in this species (78, 79). Nutrient overload leading to hyperglycemia further reduced the threshold for insulin release and the half-maximal stimulatory concentration of glucose. A similar leftward-shift of the glucose

concentration-response curve was obtained for proinsulin biosynthesis (39). Taken together, detailed analysis of beta-cell function in *P. obesus* islets showed increased sensitivity to glucose, which was further augmented in the diabetic state. This results in a high turnover of the insulin produced by the beta-cells.

Biochemical analysis showed increased glucose phosphorylation in islets of diabetic compared to normoglycemic *P. obesus* or to insulin sensitive rats, resulting from increased activity of glucokinase and hexokinase (79). Glucose oxidation was also increased in diabetic *P. obesus* leading to accelerated rise in cytosolic calcium, mitochondrial membrane hyperpolarization, NADPH and ATP production (13, 79). This is in line with the observed increase in glucokinase activity and glucose utilization and oxidation in response to short-term glucose infusion in rats (80), and in the Zucker diabetic fatty (ZDF) rat model of T2DM (81). These studies suggest that the beta-cell compensation (increased insulin secretion and proinsulin biosynthesis) to hyperglycemia in early-stage T2DM results from increased glucose utilization and oxidation that lead to higher mitochondrial ATP production. In addition, high-fat diet-induced insulin resistance in C57BL/6J mice was accompanied by increased mitochondrial volume and metabolism of FFAs and amino acids, supporting a role for enhanced mitochondrial metabolism in beta-cell compensation (82).

The Zucker fatty (ZF) rat harbors a leptin receptor mutation resulting in obesity, insulin resistance and hyperlipidemia without hyperglycemia. Therefore, it is an interesting model to study beta-cell compensation to insulin resistance. ZF rat islets exhibit increased glucose utilization and oxidation compared with Zucker lean (ZL) control rats, associated with increased anaplerosis *via* pyruvate carboxylase (81). Moreover, inhibition of pyruvate carboxylase markedly impaired glucose-stimulated insulin secretion (81), suggesting that augmentation of anaplerosis is involved in the beta-cell compensation to insulin resistance. In addition, it was shown that FFAs synergized with GLP-1 and cAMP to augment glucose-stimulated insulin secretion (24). Both esterification and oxidation of FFAs were increased in ZF compared to ZL rats, suggestive of enhanced triglyceride-fatty acid cycling. Inhibition of FFA oxidation prevented the effect of FFAs on glucose-stimulated insulin secretion. The interaction between FFAs, glucose and incretins and coordinated fatty acid-triglyceride cycling are thus important for beta-cell compensation to insulin resistance (24).

In summary, beta-cell compensation to insulin resistance and hyperglycemia is characterized by increased glucose and FFA metabolism with increased glucose utilization and oxidation, enhanced metabolism *via* anaplerotic pathways and increased FFA esterification and -oxidation. These pathways synergize with incretins to further amplify insulin release by increasing the cellular levels of signals, such as ATP that foster insulin secretion and production.

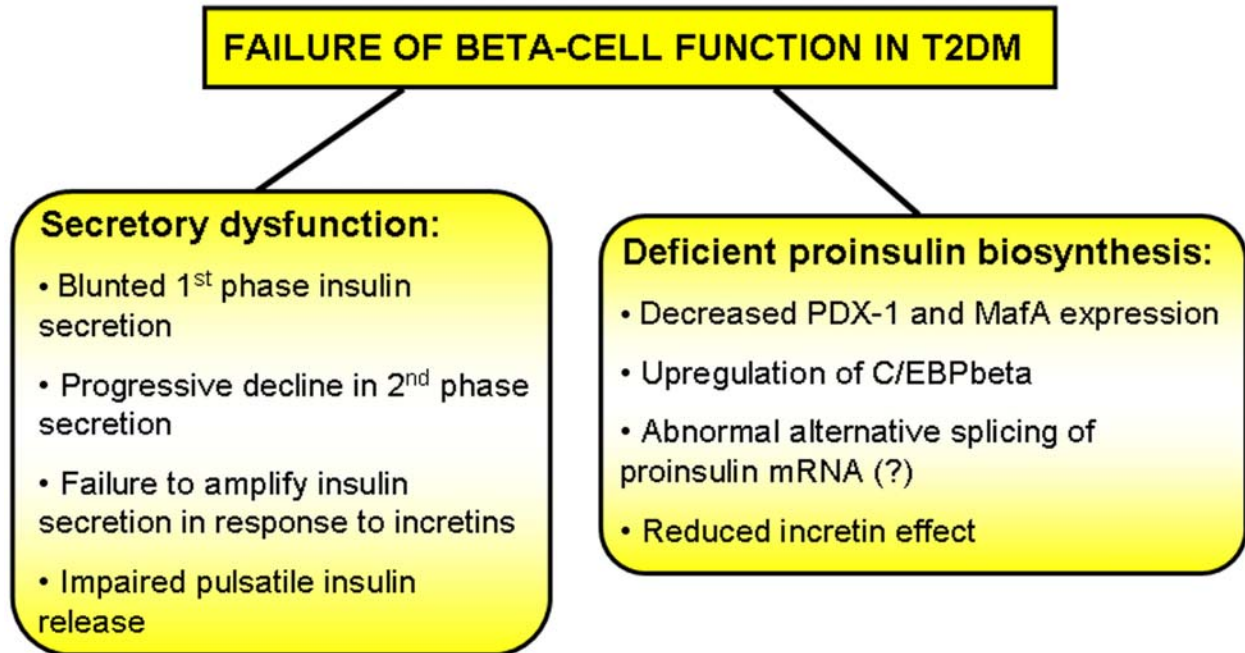


Figure 2. Processes involved in beta-cell dysfunction in type 2 diabetes.

4.3. Signaling pathways involved in beta-cell compensation

Nutrients increase beta-cell mass *via* activation of the insulin/IGF1 receptor-PI3 kinase-Akt pathway. This pathway can be stimulated by the secreted insulin, growth factors and GLP-1. Its prime role in beta-cell mass compensation was demonstrated in knockout mice for insulin/IGF1 receptors or for IRS2 in the beta-cells, which failed to increase beta-cell mass and developed diabetes (83-85). Studies in the ZF rat showing that Akt is activated in the islets, further supported the hypothesis that this pathway mediates the expansion of beta-cells in insulin resistant animals (86). In *P. obesus*, there is a marked increase of IRS2 during the early stages of diabetes, which is accompanied by increased beta-cell proliferation and up to 50% increase of beta-cell mass (87). Increased Akt phosphorylation activates mTOR, which regulates protein synthesis and beta-cell growth, proliferation and apoptosis (87). In S6K1 (downstream target of mTOR) knockout mice, beta-cell size is reduced, accompanied by hypoinsulinemia and glucose intolerance despite increased insulin sensitivity (88, 89). In diabetic *P. obesus*, inhibition of mTOR/S6K resulted in a marked increase of beta-cell apoptosis with reduced beta-cell mass, indicating that the mTOR/S6K pathway is essential for beta-cell survival under hyperglycemia in this model (87). Taken together, these studies suggest that mTOR is an important regulator of beta-cell mass and function.

mTOR is regulated by insulin/IGF-1 and nutrients through modulation of cellular ATP levels (66, 90); the latter inhibit AMPK with activation of mTOR as a consequence (32, 34). Moreover, activation of AMPK by metformin or AICAR was shown to inhibit mTOR (38). The coupling between mTOR and AMPK and their

modulation by nutrients suggest that interactions between these kinases participate in nutrient regulation of beta-cell mass.

In summary, metabolic signals provided by nutrient metabolism and different kinases, mainly those of the insulin/IGF1 receptor cascade, generate a complex signaling network that acts to enhance beta-cell function and expand beta-cell mass under conditions of nutrient overload.

5. BETA-CELL FAILURE IN TYPE 2 DIABETES

Failure to compensate for insulin resistance by increased functional beta-cell mass occurs both in humans and in animal models of type 2 diabetes (reviewed in (91)). Among the major factors driving beta-cell maladaptation are: a. extended metabolic insults, i.e. elevated levels of glucose and lipids, b. increased inflammatory mediators, and c. amyloid deposits in the islets (in humans), all augmenting the cellular stress that drives progressive beta-cell demise.

5.1. Beta-cell secretory dysfunction

A poor pancreatic secretory response is a mandatory condition for a full manifestation of T2DM (Figure 2) Impaired insulin secretion in T2DM is initially evident as blunted early (first phase) insulin response, followed by a progressive decline in the sustained (late or second phase) response to glucose (92). Although the sensitivity to non-nutrient secretagogues may seem normal at the initial stages of T2DM, the secretory response to potentiators of glucose-induced insulin secretion is attenuated when tested at similar levels of glycemia (93, 94). The defective insulin secretion in T2DM is also

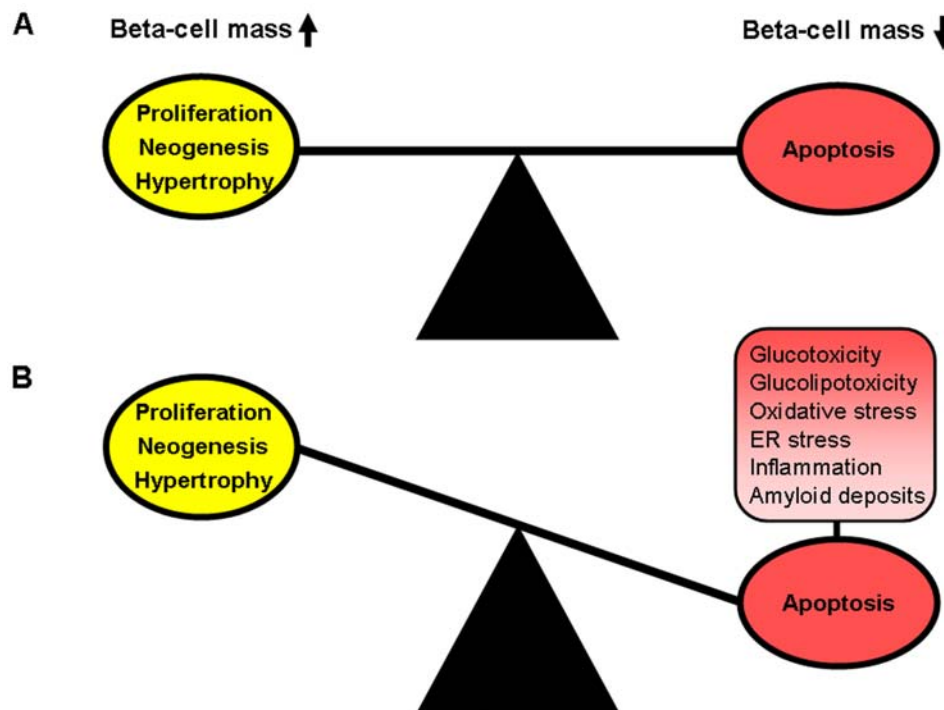


Figure 3. Regulation of beta-cell mass in normal physiology (A) and in type 2 diabetes (B)

characterized by impaired pulsatile insulin release associated with lack of postprandial glucagon suppression and inappropriate hepatic glucose production (95, 96); reduced glucose responsiveness of the beta-cells and the loss of the normal pattern of insulin secretion (first phase and pulsatility) compromise glucose homeostasis.

5.2. Deficient proinsulin biosynthesis

In vivo studies in animal models of diabetes demonstrated deleterious effects of the diabetic environment on insulin production (39, 50), resulting from decreased expression and activity of transcription factors required for proinsulin gene transcription (Figure 2). In the ZDF and 90% pancreatectomized rat models of T2DM the expression of PDX-1 and MafA was reduced, resulting in decreased proinsulin mRNA levels (50, 97-99). Normalization of blood glucose levels in ZDF rats restored PDX-1 and proinsulin gene expression (97). C/EBPβ, a member of the leucine zipper family of transcription factors that specifically represses insulin gene transcription was upregulated in the islets of both models (50). Studies in the *P. obesus* model of nutrition-induced diabetes, in which the conserved form of PDX-1 could not be detected (100), documented a time-dependent reduction of steady-state proinsulin mRNA levels during progression of nutrition-induced diabetes (101). Thus, differential regulation of various transcription factors appears to be involved in the impairment of proinsulin gene transcription during diabetes progression. Failure to increase insulin gene expression hinders the replenishment of pancreatic insulin stores under the metabolic environment of T2DM (100, 101). In addition to its effect on proinsulin gene transcription, PDX-1 affects GLUT-2 gene transcription (99), mitochondrial

metabolism (102) and pancreatic convertases (103). Therefore, decreased PDX-1 expression might also impair glucose sensing and metabolism in the beta-cell, as well as proinsulin processing.

5.3. Decreased beta-cell mass

Studies in animal models of diabetes (e.g. the obese diabetic Zucker *fa/fa* rats (104, 105)) and in autopsy material from individuals with T2DM (67, 106, 107) found a decrease in beta-cell mass that could contribute to the inability to compensate for the increased metabolic load associated with insulin resistance and obesity (Figure 3). Whereas this idea is not accepted by all authors (108), studies using large series of pancreases from patients with T2DM matched by appropriate controls documented reduced relative beta-cell volume in humans with both impaired fasting glucose and established T2DM (67). The finding of reduced beta-cell volume already in individuals with impaired fasting glucose suggests that this could have pathophysiological importance. The reduced beta-cell mass is mostly due to enhanced rate of beta-cell apoptosis, rather than inability of the beta-cells to proliferate (67, 104). Deng *et al* observed that in addition to a marked reduction in the recovery of islets, the islets of T2DM donors were functionally defective (109).

In *P. obesus*, during the early stage of diabetes there was an increased rate of beta-cell turnover (higher rates of proliferation and apoptosis) with a relatively small increase of beta-cell mass (87, 110). During disease progression a gradual increase in β-cell apoptosis was observed, resulting in a marked decrease in beta-cell mass at the end-stage of the disease (39, 110). The rapid

development of hyperglycemia in the absence of reduced beta-cell mass in the early stage of diabetes in *P. obesus* suggests that the beta-cell mass is of minor importance in the establishment of diabetes in this species, but may contribute to disease progression over-time.

6. FACTORS DRIVING BETA-CELL FAILURE

In T2DM, beta-cell loss results from increased apoptosis (Figure 3). Factors driving beta-cell dysfunction and apoptosis include: glucotoxicity, glucolipotoxicity, proinflammatory mediators, and deposits of islet amyloid polypeptide.

6.1. Glucotoxicity

Beta-cell mass and function are negatively affected by the hyperglycemic environment, a condition referred to as glucotoxicity (111). This is characterized by impairment of glucose sensing and insulin secretion, reduced expression of transcription factors important for adequate proinsulin gene transcription and increased beta-cell apoptosis (reviewed in (112)). These impairments could appear early in the course of T2DM, before persistent hyperglycemia is evident, in response to transient postprandial hyperglycemic excursions (113). It appears that both the magnitude and the duration of hyperglycemia are important for manifestation of beta-cell glucotoxicity. Indeed, reversal of hyperglycemia in both animal models and humans with T2DM resulted in at least partial recovery of beta-cell function (39, 114-116).

6.2. Glucolipotoxicity

Disorders of lipid metabolism were proposed to contribute to beta-cell dysfunction (117, 118). However, it was later shown that the toxic effect of FFAs on the pancreatic beta-cells, originally termed lipotoxicity, is evident mostly under the *in vivo* hyperglycemic environment, hence it is now referred to as glucolipotoxicity (119). Long-term exposure of rat and human pancreatic islets to high levels of FFAs and glucose impaired insulin secretion and proinsulin gene transcription and increased beta-cell apoptosis (reviewed in (6, 51)). Elevated levels of saturated but not monounsaturated fatty acids were reported to increase beta-cell apoptosis in rat and human islets (120, 121). The observed effects on beta-cell apoptosis and proinsulin gene expression induced by palmitate were suggested to be mediated by ceramide accumulation, as well as by increased partitioning into complex lipids, some of which are cytotoxic to the beta-cells (6, 21, 51). Nevertheless, inhibitory effects on insulin secretion were induced by both palmitate and oleate, and hence are unlikely to be mediated by ceramide (51). In the ZDF but not in normoglycemic ZF rats there is increased fat deposition in beta-cells, associated with apoptosis (6). In the diabetic *P. obesus* during the early phase of hyperglycemia, there was no increase in islet triglycerides or lipid deposits (112); in contrast, increased circulating lipids and beta-cell lipid deposits were observed in the very advanced stages of diabetes (39). In this model the *in vivo* beta-cell dysfunction precedes the decrease in beta-cell mass, therefore it is unlikely that the early impairment of beta-cell function in *P. obesus* is related to beta-cell steatosis.

6.3. Proinflammatory cytokines and adipokines

There is a chronic increase in inflammatory mediators in T2DM, some of which were shown to impair beta-cell function and survival (reviewed in (122-125)). *In vitro* studies in isolated human islets suggest that hyperglycemia increases beta-cell apoptosis by inducing IL-1beta (126). This was extended to the *in vivo* environment by immunohistochemical studies on pancreatic tissue from T2DM patients (126, 127). These studies showed that the effect of hyperglycemia was mediated by the activation of the Fas death pathway. In more recent studies, Donath and colleagues demonstrated a novel role for the Fas pathway in human islets, reducing insulin production and release (128).

The adipokine leptin inhibited glucose-induced insulin release and promoted beta-cell apoptosis by increasing production and release of IL-1beta and decreasing IL-1 receptor antagonist in human islets (129). Leptin, originally identified as an adipocyte-derived satiety factor, is increased in obesity-associated T2DM. Other cytokines released by adipocytes may also modulate beta-cell survival, although it is unclear if the amounts released into the circulation in obesity-related diabetes are sufficient to affect the beta-cells (125).

6.4. Islet amyloid polypeptide

Humans with T2DM are prone to develop islet amyloid deposits composed mostly of islet amyloid polypeptide (IAPP, amylin); however, its relevance to beta-cell function has been debated (130, 131). IAPP is a normal secretory product of the human beta-cell, co-secreted with insulin. The prevalence of amyloid deposits is higher in T2DM patients compared with non-diabetic individuals (67). The molecular structure of IAPP in humans, monkeys and cats allows spontaneous formation of fibrils and oligomers in an aqueous solution, which might be toxic to the beta-cells. It appears that the structure of the IAPP oligomers determines their toxicity to human islets (130, 131). Recent studies from Butler and colleagues suggest that toxic human IAPP oligomers rather than IAPP fibers initiate beta-cell apoptosis and thus contribute to the decrease of beta-cell mass in T2DM (132). Moreover, *in vitro* exposure of human islets to IAPP was shown to disrupt islet morphology and glucose mediated insulin secretion through an effect on the cell membrane (133).

7. DERANGED CELLULAR PATHWAYS INVOLVED IN BETA-CELL FAILURE IN TYPE 2 DIABETES

Several cellular stress pathways are deranged in T2DM driving beta-cell apoptosis and reducing functional beta-cell mass (Figure 3). These include: inflammatory, oxidative and endoplasmic reticulum stress.

7.1. Inflammatory stress

Activation of the innate immune system has been reported in obesity, insulin resistance and obesity-associated T2DM. These conditions are characterized by increased circulating levels of acute phase proteins as well as cytokines and chemokines (reviewed in (122-124)). The

possibility that some of the inflammatory changes might be a consequence of T2DM has been extensively investigated. Metabolic stress resulting from high caloric intake, with increased circulating levels of glucose and FFAs, appears to contribute to the loss of functional beta-cell mass in T2DM mainly by apoptosis (67). Culture of human islets with high glucose on extracellular matrix derived from bovine corneal endothelial cells stimulated IL-1 β synthesis and secretion and promoted Fas-triggered beta-cell apoptosis, in part by activating the transcription factor NF κ B (126). IL-1 β may also be produced by macrophages infiltrating the islets of patients and animal models of T2DM (134). IL-1 β expression was demonstrated in pancreatic tissue of T2DM patients, but not in non-diabetic controls (126). Increased IL-1 β expression was observed in islets of diabetic *P. obesus* fed a high calorie diet; this increase was reversible by normalization of glycemia (126). Additional studies showed that IL-1 receptor antagonist fluctuated inversely with changes in glycemia both in human and *P. obesus* beta-cells (129), suggesting that it is a physiological regulator of beta-cell viability. Small interfering RNA sequences directed against IL-1 receptor antagonist increased beta-cell apoptosis in cultured human islets even at basal glucose levels (129). Indeed, antagonism of IL-1 in patients with T2DM by administration of recombinant human IL-1 receptor antagonist resulted in improved glycemic control and beta-cell secretory function and reduced systemic inflammation (135). It should be noted, however, that although substantial experimental data support the involvement of the innate immune system in beta-cell maladaptation in T2DM, this is not accepted by all investigators in the field (136-138).

7.2. Oxidative stress

Oxidative stress appears to be an important cause of beta-cell dysfunction in T2DM via its inhibitory effects on insulin secretion and beta-cell survival. Glucotoxicity and glucolipotoxicity, which drive diabetes progression, are mediated at least in part by excess generation of ROS and other free radicals (reviewed in (139, 140)). Recent studies showed that administration of intralipid or high glucose induced oxidative stress in rat islets *in vivo* (141). ROS production in beta-cells, as in other cells, could be generated through several molecular processes: the electron transport chain in the mitochondrial membrane (142), the plasma membrane NADPH oxidase (143), non-enzymatic glycation (144), and the hexosamine biosynthetic pathway (145). ROS production in mitochondria and by the hexosamine pathway results from increased metabolic flux. Studies in isolated islets have shown that elevated glucose, palmitate or IL-1 β increased NADPH oxidase activity and its p47^{phox} subunit production (143), contributing to free radical load in the islets. Increased mitochondrial metabolic flux results in increased superoxide, activating uncoupling protein-2 (UCP2) (146). In addition, UCP2 expression is upregulated by high glucose and FFAs (141). UCP2 activation, while protecting the beta-cells from increased ROS production, uncouples glucose oxidation from ATP production, thus decreasing glucose-stimulated insulin secretion. Although oxidative stress is provoked in different tissues, beta-cells are particularly vulnerable to it

because of low expression of the main antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase (147). In addition to antioxidant enzymes, several antioxidant systems participate in maintaining the redox state of the cells, among which are the thiol antioxidants glutathione (GSH) and thioredoxin (TRX) (reviewed in (148-150)). The thioredoxin system, in addition to its role in maintaining the cellular redox state, affects beta-cell function and survival by controlling several transcription factors involved in cell proliferation and apoptosis (151). In this regard, it was recently shown that glucose induces an endogenous inhibitor of TRX, thioredoxin interacting protein (TXNIP), leading to increased beta-cell apoptosis (152). Moreover, it was recently shown that islets derived from HcB-19 mice harboring an inactivating mutation in the *Txnip* gene are protected from glucose-induced apoptosis (153). Thus, the deleterious effects of the diabetic environment on the beta-cell are driven by a chronic increase in the production of free radicals, together with diminished intracellular “redox buffering” capacity, substantiated primarily by GSH and TRX. Given the important role of the cellular redox state in signal transduction, enzyme activation, DNA and RNA synthesis, cell proliferation, differentiation and apoptosis, the shift of this balance by overproduction of ROS could represent a common pathway driving beta-cell demise in T2DM. This may explain the beneficial effects of antioxidant molecules on beta-cell function, observed both *in vitro* and in animal models of T2DM *in vivo* (154, 155).

7.3. Endoplasmic reticulum stress

Endoplasmic reticulum (ER) stress results from increased levels of misfolded proteins in the ER. This could be provoked during periods of increased biosynthetic activity when chaperones in the ER are overloaded and the ER fails to fold correctly and export the newly synthesized proteins (156). ER stress leads to activation of various pathways aimed at adaptation and restoration of normal ER function. When the ER stress response, termed unfolded protein response (UPR), fails to restore adequate ER function (by translation attenuation, degradation of misfolded proteins or increased protein folding capacity through increased transcription of ER chaperones), it turns on cell signals that lead to apoptosis (156, 157). ER stress is suggested to be involved in beta-cell apoptosis during periods of increased proinsulin biosynthesis, as in insulin resistant states and T2DM (157, 158). ER stress could also result from increased biosynthesis of IAPP, characteristic of the beta-cells in T2DM (133, 159). The UPR involves 3 major signaling pathways initiated by 3 transmembrane proteins in the ER (156, 157): IRE1 (inositol requiring 1), the pancreatic ER kinase PERK (double stranded RNA-activated protein kinase-like endoplasmic reticulum kinase) and AFT6 (activated transcription factor 6); all are maintained in an inactive conformation by association with the chaperone BiP, which dissociates upon ER stress leading to activation of proximal signal transducers. The apoptotic response to ER stress, which occurs when adaptation mechanisms fails, is initiated by PERK, IRE1 and AFT6 through the transcriptional activation of the Chop gene. A second ER stress-related apoptotic response involves activation of the c-Jun N-terminal kinase (JNK)

pathway, which is downstream to IRE1. The third ER stress pathway leading to apoptosis results from perturbation of the ER Ca^{2+} pool with activation of calpain in the cytosol. Calpain converts Caspase-12 to its active form, initiating a caspase cascade that results in apoptosis. The earliest clue to the involvement of ER stress in diabetes was obtained from studies on the Akita mouse model of spontaneous diabetes, which is associated with reduced beta-cell mass (160). The Akita mouse, a model of autosomal dominant diabetes, harbors a mutation in the proinsulin 2 gene that precludes formation of one of the disulfide bonds. To support the hypothesis that ER stress is the cause of the reduced beta-cell mass in the Akita mouse, the Akita mutation was introduced into Chop knockout mice, which resulted in delayed hyperglycemia and preservation of beta-cell mass (161). It was recently shown that the mutant proinsulin in complex with native proinsulin was trapped in the ER, impairing trafficking of insulin. This trapping mechanism decreases insulin production followed by beta-cell apoptosis, leading to hyperglycemia (162). Additional studies in animal models and *in vitro* studies in beta-cell lines provided further support for the role of ER stress in beta-cell failure in T2DM and insulin resistance (reviewed in (163)). The relevance of ER stress to human diabetes is demonstrated by the finding that a loss-of-function mutations in the *Wfs1* gene, which encodes an ER transmembrane protein, leads to ER stress, beta-cell apoptosis and hyperglycemia (164, 165).

8. CONCLUDING COMMENTS AND CLINICAL IMPLICATIONS

Beta-cell compensation for increased demand is driven by enhanced glucose, FFA and possibly amino acid metabolism. This is due to increased availability of substrates for glycolysis and TCA cycle oxidation, and increased activity of enzymes that generate signals for insulin secretion and biosynthesis. Increased nutrient supply and cellular energy (ATP) turns on signaling networks that activate mTOR/S6K1 and Akt/PKB, leading to nuclear exclusion of FOXO1. This augments beta-cell proliferation and reduces apoptosis leading to expansion of beta-cell mass as a consequence. However, protracted nutrient stimulation and accumulation of metabolic intermediates, such as acyl CoAs and ROS, and of IAPP, induce various pathways of cellular stress. This in turn impedes beta-cell function and induces apoptosis. Thus, enhanced activation of physiological adaptive mechanisms aimed to augment insulin secretion may eventually lead to beta-cell failure and reduced beta-cell mass. We hypothesize that “efficient” beta-cells which robustly augment insulin secretion in response to increased demand exhibit high metabolic and biosynthetic activity and are therefore at a higher risk for oxidative and ER stress.

This paradigm has important clinical implications for the prevention and treatment of T2DM. Treatments that reduce the secretory load exerted on the beta-cell, including reduction of caloric intake and increased physical activity, medical treatments aimed at reducing insulin resistance (e.g., metformin, glitazones), or modifiers of nutrient absorption (e.g., acarbose, orlistat), not only reduce

glycemia, but may also attenuate cellular stress allowing for better preservation of beta-cell mass and function over-time. This is supported by clinical trials showing that such treatments reduced the incidence of T2DM in patients with impaired glucose tolerance who are at high risk for developing the disease (166). On the other hand, insulin secretagogues, such as sulfonylureas, while amplifying insulin secretion may ultimately compromise beta-cell survival and function. Indeed, *in vitro* studies showed that treatment of human islets with sulfonylureas increased beta-cell apoptosis (167). Moreover, in the ADOPT study, treatment of T2DM patients with sulfonylureas was associated with a faster decline of beta-cell function compared to metformin or glitazones (168). In line with this concept, treatment with the K^+ -ATP-channel opener diazoxide enhanced the recovery of beta-cell mass following 90% pancreatectomy in rats (169). Additionally, diazoxide that allows beta-cell “rest” improved the beta-cell function of patients with T2DM (91). On the other hand, recent studies suggest that certain sulfonylureas, such as gliclazide, could have antioxidant activity and therefore may protect the beta-cell against oxidative stress (170). Thus, the potential deleterious effect of sulfonylureas on beta-cell viability and function is controversial. It should be noted that the most potent stimulus augmenting beta-cell work in the diabetic state is hyperglycemia, therefore, reducing hyperglycemia independent of the mode of therapy may protect the beta-cell in T2DM (171).

Novel treatments aimed to modify the course of T2DM should focus on better preservation of beta-cell function and mass. This can be accomplished by reducing the “work load” exerted on the beta-cells and by developing new therapeutic approaches to protect the islets from cellular stress.

9. REFERENCES

1. Kahn B.B.: Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance. *Cell* 92, 593-596 (1998)
2. Rhodes C.J.: Type 2 diabetes-a matter of beta-cell life and death? *Science* 307, 380-384 (2005)
3. Florez J.C., K.A. Jablonski, N. Bayley, T.I. Pollin, P.I.W. de Bakker, A.R. Shuldiner, W.C. Knowler, D.M. Nathan & D. Altshuler for the Diabetes Prevention Program Research Group: CF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355, 241-250 (2006)
4. Grant S.F., G. Thorleifsson, I. Reynisdottir, R. Benediktsson, A. Manolescu, J. Sainz, A. Helgason, H. Stefansson, V. Emilsson, A. Helgadóttir, U. Styrkarsdóttir, K.P. Magnusson, G.B. Walters, E. Palsdóttir, T. Jonsdóttir, T. Gudmundsdóttir, A. Gylfason, J. Saemundsdóttir, R.L. Wilensky, M.P. Reilly, D.J. Rader, Y. Bagger, C. Christiansen, V. Gudnason, G. Sigurdsson, U. Thorsteinsdóttir, J.R. Gulcher, A. Kong & K. Stefansson: Variant of transcription factor 7-like 2 (*TCF7L2*) gene

- confers risk of type 2 diabetes. *Nat Genet* 38, 320-323 (2006)
5. Sladek R., G. Rocheleau, J. Rung, C. Dina, L. Shen, D. Serre, P. Boutin, D. Vincent, A. Belisle, S. Hadjadj, B. Balkau, B. Heude, G. Charpentier, T.J. Hudson, A. Montpetit, A.V. Pshezhetsky, M. Prentki, B.I. Posner, D.J. Balding, D. Meyre, C. Polychronakos & P. Froguel: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445, 881-885 (2007)
 6. Prentki M. & C.J. Nolan: Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116, 1802-1812 (2006)
 7. Weyer C., C. Bogardus, D.M. Mott & R.E. Pratley: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104, 787-794 (1999)
 8. Poitout V. & R.P. Robertson: Glucolipotoxicity: Fuel excess and beta-cell dysfunction. *Endocr Rev* in press (2008)
 9. Maechler P. & C.B. Wollheim: Mitochondrial function in normal and diabetic beta-cells. *Nature* 414, 807-812 (2001)
 10. Ashcroft F.M., D.E. Harrison & S.J. Ashcroft: Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature* 312, 446-448 (1984)
 11. Cook D.L. & C.N. Hales: Intracellular ATP directly blocks K⁺ channels in pancreatic B-cells. *Nature* 311, 271-273 (1984)
 12. Maechler P.: Mitochondria as the conductor of metabolic signals for insulin exocytosis in pancreatic beta-cells. *Cell Mol Life Sci* 59, 1803-1818 (2002)
 13. Henquin J.C., N. Ishiyama, M. Nenquin, M.A. Ravier & J.C. Jonas: Signals and pools underlying biphasic insulin secretion. *Diabetes* 51 (Suppl 1), S60-67 (2002)
 14. Pi J., Y. Bai, Q. Zhang, V. Wong, L.M. Floering, K. Daniel, J.M. Reece, J.T. Deeney, M.E. Andersen, B.E. Corkey & S. Collins: Reactive oxygen species as a signal in glucose-stimulated insulin secretion. *Diabetes* 56, 1783-1791 (2007)
 15. Farfari S., V. Schulz, B. Corkey & M. Prentki: Glucose-regulated anaplerosis and cataplerosis in pancreatic beta-cells: possible implication of a pyruvate/citrate shuttle in insulin secretion. *Diabetes* 49, 718-726 (2000)
 16. Newgard C.B., D. Lu, M.V. Jensen, J. Schissler, A. Boucher, S. Burgess & A.D. Sherry: Stimulus/secretion coupling factors in glucose-stimulated insulin secretion: insights gained from a multidisciplinary approach. *Diabetes* 51 (Suppl 3), S389-393 (2002)
 17. MacDonald M.J., L.A. Fahien, J.D. Buss, N.M. Hasan, M.J. Fallon & M.A. Kendrick: Citrate oscillates in liver and pancreatic beta cell mitochondria and in INS-1 insulinoma cells. *J Biol Chem* 278, 51894-51900 (2003)
 18. Ronnebaum S.M., O. Ilkayeva, S.C. Burgess, J.W. Joseph, D. Lu, R.D. Stevens, T.C. Becker, A.D. Sherry, C.B. Newgard & M.V. Jensen: A pyruvate cycling pathway involving cytosolic NADP-dependent isocitrate dehydrogenase regulates glucose-stimulated insulin secretion. *J Biol Chem* 281, 30593-30602 (2006)
 19. Pongratz R.L., R.G. Kibbey, G.I. Shulman & G.W. Cline: Cytosolic and mitochondrial malic enzyme isoforms differentially control insulin secretion. *J Biol Chem* 282, 200-207 (2007)
 20. Attali V., M. Parnes, Y. Ariav, E. Cerasi, N. Kaiser, and G. Leibowitz: Regulation of insulin secretion and proinsulin biosynthesis by succinate. *Endocrinology* 147, 5110-5118 (2006)
 21. Prentki M., E. Joly, W. El-Assaad & R. Roduit: Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes* 51 (Suppl 3), S405-413 (2002)
 22. Liu Y.Q., J.A. Moibi & J.L. Leahy: Chronic high glucose lowers pyruvate dehydrogenase activity in islets through enhanced production of long chain acyl-CoA: prevention of impaired glucose oxidation by enhanced pyruvate recycling through the malate-pyruvate shuttle. *J Biol Chem* 279, 7470-7475 (2004)
 23. Magnan C., S. Collins, M.F. Berthault, N. Kassis, M. Vincent, M. Gilbert, L. Penicaud, A. Ktorza, and F. Assimacopoulos-Jeannet: Lipid infusion lowers sympathetic nervous activity and leads to increased beta-cell responsiveness to glucose. *J Clin Invest* 103, 413-419 (1999)
 24. Nolan C.J., J.L. Leahy, V. Delghingaro-Augusto, J. Moibi, K. Soni, M.L. Peyot, M. Fortier, C. Guay, J. Lamontagne, A. Barbeau, E. Przybytkowski, E. Joly, P. Masiello, S. Wang, G.A. Mitchell & M. Prentki: Beta cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling. *Diabetologia* 49, 2120-2130 (2006)
 25. Gilon P. & J.C. Henquin: Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* 22, 565-604 (2001)
 26. Drucker D.J.: The biology of incretin hormones. *Cell Metab* 3, 153-165 (2006)
 27. Gilon P. & J.C. Henquin: Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* 22, 565-604 (2001)
 28. Itoh Y., Y. Kawamata, M. Harada, M. Kobayashi, R. Fujii, S. Fukusumi, K. Ogi, M. Hosoya, Y. Tanaka, H. Uejima, H. Tanaka, M. Maruyama, R. Satoh, S. Okubo, H. Kizawa, H. Komatsu, F. Matsumura, Y. Noguchi, T. Shinohara, S. Hinuma, Y. Fujisawa & M. Fujino: Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 422, 173-176 (2003)

29. Steneberg P., N. Rubins, R. Bartoov-Shifman, M.D. Walker & H. Edlund: The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab* 1, 245-258 (2005)
30. Roduit R., C. Nolan, C. Alarcon, P. Moore, A. Barbeau, V. Delghingaro-Augusto, E. Przybykowski, J. Morin, F. Masse, B. Massie, N. Ruderman, C. Rhodes, V. Poitout, & M. Prentki: A role for the malonyl-CoA/long-chain acyl-CoA pathway of lipid signaling in the regulation of insulin secretion in response to both fuel and nonfuel stimuli. *Diabetes* 53, 1007-1019 (2004)
31. Prentki M. & B.E. Corkey: Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45, 273-283 (1996)
32. Carlin, D.: The AMP-activated protein kinase cascade - a unifying system for energy control. *Trends Biochem Sci* 29, 18-24 (2004)
33. Ruderman N. & M. Prentki: AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov* 3, 340-351 (2004)
34. Hardie D.G., S.A. Hawley & J.W. Scott: AMP-activated protein kinase - development of the energy sensor concept. *J Physiol* 574, 7-15 (2006)
35. Xue B. & B.B. Kahn: AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. *J Physiol* 574, 73-83 (2006)
36. Leclerc I., W.W. Woltersdorf, G. da Silva Xavier, R.L. Rowe, S.E. Cross, G.S. Korbitt, R.V. Rajotte, R. Smith & G.A. Rutter: Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion. *Am J Physiol Endocrinol Metab* 286, E1023-1031 (2004)
37. Eto K., T. Yamashita, J. Matsui, Y. Terauchi, M. Noda, and T. Kadowaki: Genetic manipulations of fatty acid metabolism in beta-cells are associated with dysregulated insulin secretion. *Diabetes* 51 (Suppl 3), S414-420 (2002)
38. Gleason C.E., D. Lu, L.A. Witters, C.B. Newgard & M.J. Birnbaum: The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. *J Biol Chem* 282, 10341-10351 (2007)
39. Kaiser N., R. Nesher, M.Y. Donath, M. Fraenkel, V. Behar, C. Magnan, A. Ktorza, E. Cerasi & G. Leibowitz: *Psammomys obesus*, a model for environment-gene interactions in type 2 diabetes. *Diabetes* 54 (Suppl 2), S137-144 (2005)
40. Leibowitz G., A.I. Oprescu, G. Uckaya, D.J. Gross, E. Cerasi & N. Kaiser: Insulin does not mediate glucose stimulation of proinsulin biosynthesis. *Diabetes* 52, 998-1003 (2003)
41. Wicksteed B., C. Alarcon, I. Briaud, M.K. Lingohr & C.J. Rhodes: 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. *J Biol Chem* 278, 42080-42090 (2003)
42. Wicksteed B., Y. Uchizono, C. Alarcon, J.F. McCuaig, A. Shalev & C.J. Rhodes: A cis-element in the 5' untranslated region of the preproinsulin mRNA (ppIGE) is required for glucose regulation of proinsulin translation. *Cell Metab* 5, 221-227 (2007)
43. Minn A.H., M. Kayton, D. Lorang, S.C. Hoffmann, D.M. Harlan, S.K. Libutti & A. Shalev: Insulinomas and expression of an insulin splice variant. *Lancet* 363, 363-367 (2004)
44. Minn A.H., H. Lan, M.E. Rabaglia, D.M. Harlan, B.A. Peculis, A.D. Attie & A. Shalev: Increased insulin translation from an insulin splice-variant overexpressed in diabetes, obesity, and insulin resistance. *Mol Endocrinol* 19, 794-803 (2005)
45. Leibowitz G., M.Z. Khaldi, A. Shauer, M. Parnes, A.I. Oprescu, E. Cerasi, J.C. Jonas & N. Kaiser: Mitochondrial regulation of insulin production in rat pancreatic islets. *Diabetologia* 48, 1549-1559 (2005)
46. Skelly R.H., L.C. Bollheimer, B.L. Wicksteed, B.E. Corkey & C.J. Rhodes: 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. *Biochem J* 331, 553-561 (1998)
47. Alarcon C., B. Wicksteed, M. Prentki, B.E. Corkey & C.J. Rhodes: Succinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation. *Diabetes* 51, 2496-2504 (2002)
48. Welsh M., D.A. Nielsen, A.J. MacKrell & D.F. Steiner: Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J Biol Chem* 260, 13590-13594 (1985)
49. Melloul D., S. Marshak & E. Cerasi: Regulation of insulin gene transcription. *Diabetologia* 45, 309-326 (2002)
50. Seufert J., G.C. Weir & J.F. Habener: Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein beta and transactivator islet duodenum homeobox-1 in rat pancreatic beta cells during the development of diabetes mellitus. *J Clin Invest* 101, 2528-2539 (1998)
51. Poitout V., D. Hagman, R. Stein, I. Artner, R.P. Robertson & J.S. Harmon: Regulation of the insulin gene by glucose and fatty acids. *J Nutr* 136, 873-876 (2006)

52. Dickson L.M. & C.J. Rhodes: Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? *Am J Physiol Endocrinol Metab* 287, E192-198 (2004)
53. Bonner-Weir S., L.A. Baxter, G.T. Schuppert & F.E. Smith: A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. *Diabetes* 42, 1715-1720 (1993)
54. Hayek A., G.M. Beattie, V. Cirulli, A.D. Lopez, C. Ricordi & J.S. Rubin: Growth factor/matrix-induced proliferation of human adult beta-cells. *Diabetes* 44, 1458-1460 (1995)
55. Dor Y., J. Brown, O.I. Martinez & D.A. Melton: Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429, 41-46 (2004)
56. Nir T., D.A. Melton & Y. Dor: Recovery from diabetes in mice by beta cell regeneration. *J Clin Invest* 117, 2553-2561 (2007)
57. Teta M., M.M. Rankin, S.Y. Long, G.M. Stein & J.A. Kushner: Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell* 12, 817-826 (2007)
58. Xu X., J. D'Hoker, G. Stangé, S. Bonn , N. De Leu, X. Xiao, M. Van De Casteele, G. Mellitzer, Z. Ling, D. Pipeleers, L. Bouwens, R. Scharfmann, G. Gradwohl & H. Heimberg: Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 132, 197-207 (2008)
59. Manning B.D. & L.C. Cantley: AKT/PKB signaling: Navigating downstream. *Cell* 129, 1261-1274 (2007)
60. Withers D.J., J.S. Gutierrez, H. Towery, D.J. Burks, J.M. Ren, S. Previs, Y. Zhang, D. Bernal, S. Pons, G.I. Shulman, S. Bonner-Weir & M.F. White: Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391, 900-904 (1998)
61. Briaud I., L.M. Dickson, M.K. Lingohr, J.F. McCuaig, J.C. Lawrence & C.J. Rhodes: Insulin receptor substrate-2 proteasomal degradation mediated by a mammalian target of rapamycin (mTOR)-induced negative feedback down-regulates protein kinase B-mediated signaling pathway in beta-cells. *J Biol Chem* 280, 2282-2293 (2005)
62. Lingohr M.K., I. Briaud, L.M. Dickson, J.F. McCuaig, C. Alarcon, B.L. Wicksteed & C.J. Rhodes: Specific regulation of IRS-2 expression by glucose in rat primary pancreatic islet beta-cells. *J Biol Chem* 281, 15884-15992 (2006)
63. Park S., X. Dong, T.L. Fisher, S. Dunn, A.K. Omer, G. Weir & M.F. White: Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. *J Biol Chem* 281, 1159-1168 (2006)
64. Kitamura T., J. Nakae, Y. Kitamura, Y. Kido, W.H. Biggs, 3rd, C.V. Wright, M.F. White, K.C. Arden & D. Accili: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110, 1839-1847 (2002)
65. Boucher M.J., L. Selander, L. Carlsson & H. Edlund: Phosphorylation marks IPF1/PDX1 protein for degradation by glycogen synthase kinase 3-dependent mechanisms. *J Biol Chem* 281, 6395-6403 (2006)
66. Wullschlegel S., R. Loewith & M.N. Hall: TOR signaling in growth and metabolism. *Cell* 124, 471-484 (2006)
67. Butler A.E., J. Janson, S. Bonner-Weir, R. Ritzel, R.A. Rizza & P.C. Butler: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52, 102-110 (2003)
68. Kendall D.M., D.E. Sutherland, J.S. Najarian, F.C. Goetz & R.P. Robertson: Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. *N Engl J Med* 322, 898-903 (1990)
69. Menge B.A., A. Tannapfel, O. Belyaev, R. Drescher, C. Muller, W. Uhl, W.E. Schmidt, & J.J. Meier: Partial pancreatectomy in adult humans does not provoke beta-cell regeneration. *Diabetes* 57, 142-149 (2008)
70. Matveyenko A.V., J.D. Veldhuis & P.C. Butler: Mechanisms of impaired fasting glucose and glucose intolerance induced by an approximate 50% pancreatectomy. *Diabetes* 55, 2347-2356 (2006)
71. Frayling T.M.: Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 8, 657-662 (2007)
72. Helgason A., S. Palsson, G. Thorleifsson, S.F. Grant, V. Emilsson, S. Gunnarsdottir, A. Adeyemo, Y. Chen, G. Chen, I. Reynisdottir, R. Benediktsson, A. Hinney, T. Hansen, G. Andersen, K. Borch-Johnsen, T. Jorgensen, H. Schafer, M. Faruque, A. Doumatey, J. Zhou, R. L. Wilensky, M. P. Reilly, D.J. Rader, Y. Bagger, C. Christiansen, G. Sigurdsson, J. Hebebrand, O. Pedersen, U. Thorsteinsdottir, J.R. Gulcher, A. Kong, C. Rotimi & K. Stefansson: Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 39, 218-225 (2007)
73. Hattersley A.T.: Prime suspect: the TCF7L2 gene and type 2 diabetes risk. *J Clin Invest* 117, 2077-2079 (2007)
74. Lyssenko V., R. Lupi, P. Marchetti, S. Del Guerra, M. Orho-Melander, P. Almgren, M. Sjogren, C. Ling, K.F. Eriksson, A.L. Lethagen, R. Mancarella, G. Berglund, T. Tuomi, P. Nilsson, S. Del Prato & L. Groop: Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 117, 2155-2163 (2007)
75. Barker D.J., C.N. Hales, C.H. Fall, C. Osmond, K. Phipps & P.M. Clark: Type 2 (non-insulin-dependent)

diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36, 62-67 (1993)

76. Jimenez-Chillaron J.C., M. Hernandez-Valencia, C. Reamer, S. Fisher, A. Joszi, M. Hirshman, A. Oge, S. Walrond, R. Przybyla, C. Boozer, L.J. Goodyear, and M.E. Patti: Beta-cell secretory dysfunction in the pathogenesis of low birth weight-associated diabetes: a murine model. *Diabetes* 54, 702-711 (2005)

77. Simmons R.A., I. Suponitsky-Kroyter & M.A. Selak: Progressive accumulation of mitochondrial DNA mutations and decline in mitochondrial function lead to beta-cell failure. *J Biol Chem* 280, 28785-28791 (2005)

78. Nesher R., D.J. Gross, M.Y. Donath, E. Cerasi & N. Kaiser: Interaction between genetic and dietary factors determines beta-cell function in *Psammomys obesus*, an animal model of type 2 diabetes. *Diabetes* 48, 731-737 (1999)

79. Nesher R., N. Warwar, A. Khan, S. Efendic, E. Cerasi, and N. Kaiser: Defective stimulus-secretion coupling in islets of *Psammomys obesus*, an animal model for type 2 diabetes. *Diabetes* 50, 308-314 (2001)

80. Chen C., L. Bumbalo, and J.L. Leahy: Increased catalytic activity of glucokinase in isolated islets from hyperinsulinemic rats. *Diabetes* 43, 684-689 (1994)

81. Liu Y.Q., T.L. Jetton & J.L. Leahy: Beta-cell adaptation to insulin resistance. Increased pyruvate carboxylase and malate-pyruvate shuttle activity in islets of nondiabetic Zucker fatty rats. *J Biol Chem* 277, 39163-39168 (2002)

82. Kooptiwut S., S. Zraika, A.W. Thorburn, M.E. Dunlop, R. Darwiche, T.W. Kay, J. Proietto & S. Andrikopoulos: Comparison of insulin secretory function in two mouse models with different susceptibility to beta-cell failure. *Endocrinology* 143, 2085-2092 (2002)

83. Withers D.J., D.J. Burks, H.H. Towery, S.L. Altamuro, C.L. Flint & M.F. White: Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23, 32-40 (1999)

84. Ueki K., T. Okada, J. Hu, C.W. Liew, A. Assmann, G.M. Dahlgren, J.L. Peters, J. G. Shackman, M. Zhang, I. Artner, L.S. Satin, R. Stein, M. Holzenberger, R.T. Kennedy, C.R. Kahn & R.N. Kulkarni: Total insulin and IGF-I resistance in pancreatic beta cells causes overt diabetes. *Nat Genet* 38, 583-588 (2006)

85. Okada T., C.W. Liew, J. Hu, C. Hinault, M. D. Michael, J. Krtzfeldt, C. Yin, M. Holzenberger, M. Stoffel & R.N. Kulkarni: Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. *Proc Natl Acad Sci U S A* 104, 8977-8982 (2007)

86. Jetton T.L., J. Lausier, K. LaRock, W. E. Trotman, B. Larmie, A. Habibovic, M. Peshavaria & J.L. Leahy: Mechanisms of compensatory beta-cell growth in insulin-resistant rats: roles of Akt kinase. *Diabetes* 54, 2294-2304 (2005)

87. Fraenkel M., M. Ketzinil-Gilad, Y. Ariav, O. Pappo, M. Karaca, J. Castel, M. F. Berthault, C. Magnan, E. Cerasi, N. Kaiser, and G. Leibowitz: mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. *Diabetes* 57, 945-957 (2008)

88. Um S.H., F. Frigerio, M. Watanabe, F. Picard, M. Joaquin, M. Sticker, S. Fumagalli, P.R. Allegrini, S.C. Kozma, J. Auwerx & G. Thomas. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431, 200-205 (2004)

89. Ruvinsky I., N. Sharon, T. Lerer, H. Cohen, M. Stolovich-Rain, T. Nir, Y. Dor, P. Zisman & O. Meyuhas: Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev* 19, 2199-2211 (2005)

90. Um S.H., D. D'Alessio & G. Thomas: Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. *Cell Metab* 3, 393-402 (2006)

91. Wajchenberg B.L.: beta-cell failure in diabetes and preservation by clinical treatment. *Endocr Rev* 28, 187-218 (2007)

92. Pratley R.E. & C. Weyer: The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. 44, 929-945 (2001)

93. Ward W.K., J.C. Beard, J.B. Halter & D. Porte Jr.: Pathophysiology of insulin secretion in diabetes mellitus. *Adv Exp Med Biol* 189, 137-158 (1985)

94. Ward W.K., D.C. Bolgiano, B. McKnight, J.B. Halter & D. Porte Jr.: Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 74, 1318-1328 (1984)

95. O'Rahilly S, R.C. Turner & D.R. Matthews: Impaired pulsatile secretion of insulin in relatives of patients with non-insulin -dependent diabetes. *N Engl J Med* 318, 1225-1230 (1988)

96. Meier J.J., L.L. Kjems, J.D. Veldhuis, P. Lefebvre & P.C. Butler: Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion: further evidence for the intraislet insulin hypothesis. *Diabetes* 55, 1051-1056 (2006)

97. Harmon J.S., C.E. Gleason, Y. Tanaka, E.A. Oseid, K.K. Hunter-Berger & R.P. Robertson: *In vivo* prevention of hyperglycemia also prevents glucotoxic effects on PDX-1 and insulin gene expression. *Diabetes* 48, 1995-2000 (1999)

98. Hagman D.K., L.B. Hays, S.D. Parazzoli & V. Poitout: Palmitate inhibits insulin gene expression by altering PDX-1 nuclear localization and reducing MafA expression in isolated rat islets of Langerhans. *J Biol Chem* 280, 32413-32418 (2005)
99. Zangen D.H., S. Bonner-Weir, C.H. Lee, J.B. Latimer, C.P. Miller, J.F. Habener & G.C. Weir: Reduced insulin, GLUT2, and IDX-1 in beta-cells after partial pancreatectomy. *Diabetes* 46, 258-264 (1997)
100. Leibowitz G., S. Ferber, A. Apelqvist, H. Edlund, D.J. Gross, E. Cerasi, D. Melloul & N. Kaiser: IPF1/PDX1 deficiency and beta-cell dysfunction in *Psammomys obesus*, an animal with type 2 diabetes. *Diabetes* 50, 1799-1806 (2001)
101. Leibowitz G., G. Uçkaya, A.I. Oprescu, E. Cerasi, D.J. Gross & N. Kaiser: Glucose-regulated proinsulin gene expression is required for adequate insulin production during chronic glucose exposure. *Endocrinology* 143, 3214-3220 (2002)
102. Gauthier B.R., T. Brun, E.J. Sarret, H. Ishihara, O. Schaad, P. Descombes & C.B. Wollheim: Oligonucleotide microarray analysis reveals PDX1 as an essential regulator of mitochondrial metabolism in rat islets. *J Biol Chem* 279, 1121-1130 (2004)
103. Hart A.W., N. Baeza, A. Apelqvist, & H. Edlund: Attenuation of FGF signaling in mouse beta-cells leads to diabetes. *Nature* 14, 864-868 (2000)
104. Pick A., J. Clark, C. Kubstrup, M. Levisetti, W. Pugh, S. Bonner-Weir & K.S. Polonsky: Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 47, 358-364 (1998)
105. Topp B.G., L.L. Atkinson & D.T. Finegood: The dynamics of insulin sensitivity, beta cell function, and beta cell mass during the development of diabetes in fa/fa rats. *Am J Physiol Endocrinol Metab* 293, E1730-1735 (2007)
106. Sakuraba H., H. Mizukami, N. Yagihashi, R. Wada, C. Hanyu & S. Yagihashi: Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islets of Japanese type 2 diabetic patients. *Diabetologia* 45, 85-96 (2002)
107. Yoon K.H., S.H. Ko, J.H. Cho, J.M. Lee, Y.B. Ahn, K.H. Song, S.J. Yoo, M.I. Kang, B.Y. Cha, K.W. Lee, H.Y. Son, S.K. Kang, H.S. Kim, I.K. Lee & S. Bonner-Weir: Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 88, 2300-2308 (2003)
108. Guiot Y., C. Sempoux, P. Moulin & J. Rahier: No decrease of the beta-cell mass in type 2 diabetic patients. *Diabetes* 50 (Suppl 1), S188 (2001)
109. Deng S., M. Vatamaniuk, X. Huand, N. Doliba, N.M. Lian, A. Frank, E. Velidwdwoglu, N.M. Desai, B. Koeberlein, B. Wolf, C.F. Barker, A. Naji, F.M. Matschinsky & J.F. Markmann: Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* 53, 624-632 (2004)
110. Kaiser N., M. Yuli, G. Uckaya, A.I. Oprescu, M.F. Berthault, C. Kargar, M.Y. Donath, E. Cerasi & A. Ktorza: Dynamic changes in beta-cell mass and pancreatic insulin during the evolution of nutrition-dependent diabetes in *Psammomys obesus*: impact of glycemic control. *Diabetes* 54, 138-145 (2005)
111. Unger R.H. & S. Grundy: Hyperglycaemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia* 28, 119-121 (1985)
112. Kaiser N., G. Leibowitz & R. Nesher: Glucotoxicity and beta-cell failure in type 2 diabetes mellitus. *J Pediatr Endocrinol Metab* 16, 5-22 (2003)
113. Donath M.Y., J.A. Ehses, K. Maedler, D.M. Schumann, H. Ellingsgaard, E. Eddler & M. Reinecke: Mechanisms of beta-cell death in type 2 diabetes. *Diabetes* 54 (Suppl 2), S108-113 (2005)
114. Turner R.C., S.T. McCarthy, R.R. Holman & E. Harris: Beta-cell function improved by supplementing basal insulin secretion in mild diabetes. *Br Med J* 1, 1252-1254 (1976)
115. Rossetti L., G.I. Shulman, W. Zawulich & R.A. DeFronzo: Effect of chronic hyperglycemia on *in vivo* insulin secretion in partially pancreatectomized rats. *J Clin Invest* 80, 1033-1044 (1987)
116. Ilkova H., B. Glaser, A. Tunçkale, N. Bagriaçik & E. Cerasi: Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients by transient intensive insulin treatment. *Diabetes Care* 20, 1353-1356 (1997)
117. Unger R.H.: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44, 863-870 (1995)
118. Lee Y., H. Hirose, M. Ohneda, J.H. Johnson, J.D. McGarry & R.H. Unger: Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 91, 10878-10882 (1994)
119. Poitout V. & R.P. Robertson: Minireview: secondary beta-cell failure in type 2 diabetes - a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 143, 339-342 (2002)
120. Eitel K., H. Staiger, M.D. Brendel, D. Brandhorst, R.G. Bretzel, H.U. Haring, & M. Kellerer: Different role of saturated and unsaturated fatty acids in beta-cell apoptosis. *Biochem Biophys Res Commun* 299, 853-856 (2002)
121. Maedler K., G.A. Spinas, D. Dyntar, W. Moritz, N. Kaiser, and M.Y. Donath: Distinct effects of saturated and

monounsaturated fatty acids on beta-cell turnover and function. *Diabetes* 59, 69-76 (2001)

122. Donath M.Y., J. Storling, K. Maedler & T. Mandrup-Poulsen: Inflammatory mediators and beta-cell failure: a link between type 1 and type 2 diabetes. *J Mol Med* 81, 455-470 (2003)

123. Schmidt M.I. & B.B. Duncan: Diabetes: an inflammatory metabolic condition. *Clin Chem Lab Med* 41, 1120-1130 (2003)

124. Kolb H. & T. Mandrup-Poulsen: An immune origin of type 2 diabetes? *Diabetologia* 48, 1038-1050 (2005)

125. Donath M.Y., J. Storling, L.A. Berchtold, N. Billestrup & T. Mandrup-Poulsen: Cytokines and beta-cell biology: from concept to clinical translation. *Endocr Rev* in press (2008)

126. Maedler K., P. Sergeev, F. Ris, J. Oberholzer, H.I. Joller-Jemelka, G.A. Spinas, N. Kaiser, P.A. Halban, M.Y. Donath: Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110, 851-60 (2002)

127. Maedler K., G.A. Spinas, R. Lehmann, P. Sergeev, M. Weber, A. Fontana, N. Kaiser N & M.Y. Donath: Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets. *Diabetes* 50: 1683-1690 (2001)

128. Schumann D.M., K. Maedler, I. Franklin, D. Konrad, J. Storling, M. Böni-Schnetzler, A. Gjinojci, M.O. Kurrer, B.R. Gauthier, D. Bosco, A. Andres, T. Berney, M. Greter, B. Becher, A.V. Chervonsky, P.A. Halban, T. Mandrup-Poulsen, C.B. Wollheim & M.Y. Donath: The Fas pathway is involved in pancreatic beta cell secretory function. *Proc Natl Acad Sci U S A* 104, 2861-2866 (2007)

129. Maedler K., P. Sergeev, J.A. Ehses, Z. Mathe, D. Bosco, T. Berney, J.M. Daye, M. Reinecke, P.A. Halban & M.Y. Donath: Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. *Proc Natl Acad Sci U S A* (2004)

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

131. Hull R.L., G.T. Westermark, P. Westermark & S.E. Kahn: Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 89, 3629-3643 (2004)

132. Meier J.J., R. Kayed, C.Y. Lin, T. Gurlo, L. Haataja, S. Jayasinghe, R. Langen, C.G. Glabe & P.C. Butler: Inhibition of human IAPP fibril formation does not prevent beta-cell death: evidence for distinct actions of oligomers and fibrils of human IAPP. *Am J Physiol Endocrinol Metab* 291, E1317-1324 (2006)

133. Ritzel R.A., J.J. Meier, C.Y. Lin, J.D. Veldhuis & P.C. Butler: Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islet. *Diabetes* 56, 65-71 (2007)

134. Ehses J.A., A. Perren, E. Eppler, P. Ribaux, J.A. Pospisilik, R. Maor-Cahn, X. Gueripel, H. Ellingsgaard, M.K. Schneider, G. Biollaz, A. Fontana, M. Reinecke, F. Homo-Delarche & M.Y. Donath: Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* 56, 2356-2370 (2007)

135. Larsen C.M., M. Faulenbach, A. Vaag, A. Volund, J.A. Ehses, B. Seifert, T. Mandrup-Poulsen & M.Y. Donath: Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356, 1517-1526 (2007)

136. Elouil H., A.K. Cardozo, D.L. Eizirik, J.C. Henquin & J.C. Jonas: High glucose and hydrogen peroxide increase c-Myc and haeme-oxygenase 1 mRNA levels in rat pancreatic islets without activating NFkappaB. *Diabetologia* 48, 496-505 (2005)

137. Cnop M., N. Welsh, J.C. Jonas, A. Jörns, S. Lenzen & D.L. Eizirik: Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 54 (Suppl 2), S97-107 (2005)

138. Jörns A., K.J. Rath, O. Bock & S. Lenzen: Beta cell death in hyperglycaemic *Psammomys obesus* is not cytokine-mediated. *Diabetologia* 49, 2704-2712 (2006)

139. Newsholme P., E.P. Haber, S.M. Hirabara, E.L. Rebelato, J. Procopio, D. Morgan, H.C. Oliveira-Emilio, A.R. Carpinelli & R. Curi: Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *J Physiol* 583, 9-24 (2007)

140. Kaneto H., N. Katakami, D. Kawamori, T. Miyatsuka, K. Sakamoto, T.A. Matsuoka, M. Matsuhisa & Y. Yamasaki: Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxid Redox Signal* 9, 355-366 (2007)

141. Tang C., P. Han, A.I. Oprea, S.C. Lee, A.V. Gyulkhandanyan, G.N. Chan, M.B. Wheeler & A. Giacca: Evidence for a role of superoxide generation in glucose-induced beta-cell dysfunction *in vivo*. *Diabetes* 56, 2722-2731 (2007)

142. Lowell B.B. & G.I. Shulman: Mitochondrial dysfunction and type 2 diabetes. *Science* 307, 384-387 (2005)

143. Morgan D., H.R. Oliveira-Emilio, D. Keane, A.E. Hirata, M. Santos da Rocha, S. Bordin, R. Curi, P. Newsholme & A.R. Carpinelli: Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal beta cell line. *Diabetologia* 50, 359-369 (2007)

144. Ceriello A.: Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes Nutr Metab* 12, 42-46 (1999)
145. Kaneto H., G. Xu, K.H. Song, K. Suzuma, S. Bonner-Weir, A. Sharma & G.C. Weir: Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J Biol Chem* 276, 31099-31104 (2001)
146. Krauss S., C.Y. Zhang, L. Scorrano, L.T. Dalgaard, J. St-Pierre, S.T. Grey & B.B. Lowell: Superoxide-mediated activation of uncoupling protein 2 causes pancreatic beta cell dysfunction. *J Clin Invest* 112, 1831-1842 (2003)
147. Lenzen S., J. Drinkgern & M. Tiedge: Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 20, 463-466 (1996)
148. Patwari P., L.J. Higgins, W.A. Chutkow, J. Yoshioka & R.T. Lee: The interaction of thioredoxin with Txnip. Evidence for formation of a mixed disulfide by disulfide exchange. *J Biol Chem* 281, 21884-21891 (2006)
149. Valko M., C.J. Rhodes, J. Moncol, M. Izakovic & M. Mazur: Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interact* 160, 1-40 (2006)
150. Lillig C.H. & A. Holmgren: Thioredoxin and related molecules - from biology to health and disease. *Antioxid Redox Signal* 9, 25-47 (2007)
151. Minn A.H., C.A. Pise-Masison, M. Radonovich, J.N. Brady, P. Wang, C. Kendziorski & A. Shalev: Gene expression profiling in INS-1 cells overexpressing thioredoxin-interacting protein. *Biochem Biophys Res Commun* 336, 770-778 (2005)
152. Minn A.H., C. Hafele & A. Shalev: Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology* 146, 2397-2495 (2005)
153. Chen J., G. Saxena, I.N. Mungrue, A.J. Lusis & A. Shalev: Thioredoxin-interacting protein: A critical link between glucose toxicity and beta cell apoptosis. *Diabetes* 57, 938-944 (2008)
154. Kaneto H., Y. Kajimoto, J. Miyagawa, T. Matsuoka, Y. Fujitani, Y. Umayahara, T. Hanafusa, Y. Matsuzawa, Y. Yamasaki & M. Hori: Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48, 2398-2406 (1999)
155. Tanaka Y., C.E. Gleason, P.O. Tran, J.S. Harmon & R.P. Robertson: Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidant. *Proc Natl Acad Sci U S A* 96, 10857-10862 (1999)
156. Fonseca S.G., K.L. Lipson & F. Urano: Endoplasmic reticulum stress signaling in pancreatic beta-cells. *Antioxid Redox Signal* 9, 2335-2344 (2007)
157. Ortsäter H. & A. Sjöholm: A busy cell - endoplasmic reticulum stress in the pancreatic beta-cell. *Mol Cell Endocrinol* 277, 1-5 (2007)
158. Marchetti P., M. Bugliani, R. Lupi, L. Marselli, M. Masini, U. Boggi, F. Filippini, G.C. Weir, D.L. Eizirik & M. Cnop: The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia* 50, 2486-2494, 2007
159. Huang C.J., C.Y. Lin, L. Haataja, T. Gurlo, A.E. Butler, R.A. Rizza & P.C. Butler: High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. *Diabetes* 56, 2016-2027 (2007)
160. Wang J., T. Takeuchi, S. Tanaka, S.K. Kubo, T. Kayo, D. Lu, K. Takata, A. Koizumi & T. Izumi: A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. *J Clin Invest* 103, 27-37 (1999)
161. Oyadomari S., A. Koizumi, K. Takeda, T. Gotoh, S. Akira, E. Araki & M. Mori: Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest* 109, 525-532 (2002)
162. Liu M., I. Hodish, C.J. Rhodes & P. Arvan: Proinsulin maturation, misfolding, and proteotoxicity. *Proc Natl Acad Sci U S A* 104, 15841-15846 (2007)
163. Rajan S.S., V. Srinivasan, M. Balasubramanyam & U. Tatu: Endoplasmic reticulum (ER) stress & diabetes. *Indian J Med Res* 125, 411-424 (2007)
164. Inoue H., Y. Tanizawa, J. Wasson, P. Behn, K. Kalidas, E. Bernal-Mizrachi, M. Mueckler, H. Marshall, H. Donis-Keller, P. Crock, D. Rogers, M. Mikuni, H. Kumashiro, K. Higashi, G. Sobue, Y. Oka & M.A. Permutt: A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome) *Nat Genet* 20, 143-148 (1998)
165. Fonseca S.G., M. Fukuma, K.L. Lipson, L.X. Nguyen, J.R. Allen, Y. Oka & F. Urano: WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. *J Biol Chem* 280, 39609-39615 (2005)
166. Chiasson J.L.: Prevention of Type 2 diabetes: fact or fiction? *Expert Opin Pharmacother* 8, 3147-3158 (2007)
167. Maedler K., R.D. Carr, D. Bosco, R. A. Zuellig, T. Berney & M.Y. Donath: Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J Clin Endocrinol Metab* 90, 501-506 (2005)

168. Kahn S.E., S.M. Haffner, M.A. Heise, W.H. Herman, R.R. Holman, N.P. Jones, B.G. Kravitz, J.M. Lachin, M.C. O'Neill, B. Zinman & G. Viberti: Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 355, 2427-2443 (2006)

169. Leahy J.J., L.M. Bumbalo & C. Chen: Diazoxide causes recovery of beta-cell glucose responsiveness in 90% pancreatectomized diabetic rats. *Diabetes* 43: 173-179 (1994)

170. Del Guerra S., M. Grupillo, M. Masini, R. Lupi, M. Bugliani, S. Torri, U. Boggi, M. Del Chiaro, F. Vistoli, F. Mosca, S. Del Prato & P. Marchetti: Gliclazide protects human islet beta-cells from apoptosis induced by intermittent high glucose. *Diabetes Metab Res Rev* 23, 234-238 (2007)

171. Eriksson J.G., M. Lehtovirta, B. Ehrnstrom, S. Salmela & L. Groop: Long-term beneficial effects of glipizide treatment on glucose tolerance in subjects with impaired glucose tolerance. *J Intern Med* 259, 553-560 (2006)

Key Words: Beta-Cells, Beta-Cell Adaptation, Beta-Cell Failure, Insulin Secretion, Proinsulin Biosynthesis, Beta-Cell Mass, Glucotoxicity, Glucolipotoxicity, Inflammatory Stress, Endoplasmic Reticulum Stress, Oxidative Stress, Type 2 Diabetes, Review

Send correspondence to: Nurit Kaiser, Endocrinology and Metabolism service, Department of Medicine, Hadassah Hospital, P.O. Box 12000, Jerusalem 91120, Israel, Tel: 972-2-6776786, Fax: 972-2-6437940, E-mail: nuritk@hadassah.org.il

<http://www.bioscience.org/current/vol14.htm>