"The hyperstimulated β cell: prelude to diabetes?"
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Type 2 diabetes (T2D) is most often initiated by diminished sensitivity of insulin target tissues, which is normally compensated for by increased secretory activity of β cells and by expansion of the functional β-cell mass. However, with worsening of the disease mainly caused by mitochondrial dysfunction, oxidative and endoplasmic reticulum stress, and glucometabolism, this adaptive mechanism fails to compensate for the increased insulin needs, leading to insufficient hormone supply and postprandial hyperglycemia. Indeed, hyperstimulation of β cells may lead to β-cell failure and the progressive deterioration of insulin secretion accompanied by a loss of β-cell mass in predisposed subjects. Insufficient functional β-cell mass is also the underlying cause of type 1 diabetes, underscoring the importance of understanding β-cell dynamics. Knowledge of how insulin gene expression and insulin secretion are regulated and how the β cell develops defenses against metabolic stress or after injury can help to better define normal and abnormal pancreatic β-cell function. During the XIIIth symposium of the International Group on Insulin Secretion (IGIS), supported by an unrestricted educational grant from Servier, the most recent research findings from the foremost international specialists in the field of the hyperstimulated β cell and diabetes were presented. Below is a summary of the main points addressed.

I- What is the role of epigenetics in insulin gene expression and insulin secretion and action

Epigenetics literally refers to events that are above or beside genetics, that is, beyond DNA sequence alterations. In other words, an epigenetic phenomenon is a change in phenotype that is heritable, but does not involve DNA mutation. This change in phenotype must be switch-like and heritable even when the initial conditions that caused the switch disappear. The molecular view of epigenetics consists of the sum of alterations to DNA and histone proteins that collectively establish and propagate different patterns of gene expression and silencing from the same genome. Thus, DNA can be methylated and the histone tail can be subjected to many modifications such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, adenosine diphosphate (ADP) ribosylation, and proline isomerization, among others, all leading to alteration of the expression of the target gene (Figure 1). Whereas the role of DNA methylation in epigenetic regulation is well established, it remains to be seen whether
all types of histone modifications contribute to the epigenetic state. Important environmental factors that have been demonstrated to modulate DNA methylation and histone modifications include nutrition, radiation, and chemical toxins.

1. Epigenetic control of insulin gene transcription

The human insulin (INS) gene is expressed exclusively in the β cells of the pancreatic islets and is clustered with three other genes in an interval of <40 kb: tyrosine hydroxylase (TH)–insulin-like growth factor 2 antisense (IGF2AS)–insulin like growth factor 2 (IGF2). Felsenfeld’s group showed that active epigenetic modifications are distributed over the entire coding region of the INS gene in isolated human islets, causing altered expression that may occur in health and disease. Interestingly, they identified a region of ~80kb around the INS gene, which is marked by almost uniformly elevated levels of histone acetylation and H3K4 dimethylation, that could be involved in the regulation of the islet-specific coordinate expression of INS, TH, and IGF2 genes. This is of particular interest as these genes have been associated with obesity, birth size, type 1 diabetes, polycystic ovary syndrome, overgrowth in Beckwith–Wiedemann syndrome and possibly with hypertension. In addition, the INS gene was shown to physically interact with the synaptotagmin VIII (SYT8) gene located over 300 kb away, which encodes a transmembrane protein involved in the mediation of Ca^{2+} regulation of exocytosis in β cells. This interaction is allowed by the binding of the CCCTC-binding factor (CTCF) to specific DNA sequence elements, called insulator sites (Figure 2). The role of such elements is to prevent inappropriate interactions between adjacent chromatin domains and to organize the nearby genome: one type of insulator site establishes domains that separate enhancers and unrelated promoters in order to block their in-

Figure 1. Example of the diverse posttranslational methylation marks on the histone H3 tail (from reference 1: Cooper and El-Osta. Circ Res. 2010;107(12):1403-1413. © 2010, American Heart Association, Inc.). Amino acid residues are shown in different colors and (K) lysine highlighted in yellow. (A) The chemical variation illustrated for simplicity as mono- (m1), di- (m2), and tri- (m3) methylation of lysine 4 of histone H3. (B) Methylation marks are correlated with distinct gene expression patterns; for example, H3K4m3 (H3K4 trimethylation) is identified on active transcriptional start sites of gene sequences, whereas H3K9m3 is tightly associated with the suppression of gene expression and constitutive heterochromatin. Facultative heterochromatin (silent euchromatin) has distinguishing H3K27 trimethylation (H3K27m3) identified on specific gene sequences.
2. Metabolic programming of insulin secretion and action

Diet is one environmental factor that plays an important role in influencing the development and progression of T2D even early in life, and epigenetic regulation of gene expression has been implicated in mediating these programming effects of early diet. In the rat, exposure to maternal suboptimal nutrition (low protein, LP) during fetal and early postnatal life is a well-characterized model for nutritional programming of T2D. Indeed, the LP offspring undergo a loss of glucose tolerance and develop a phenotype similar to human T2D by 17 mo of age, ie, with the same age-dependent development of the phenotype as in humans. Moreover, LP offspring have alterations in the action of insulin and changes in the expression of proteins downstream of the insulin receptor in skeletal muscle (notably the phosphatidylinositol 3 [PI3] kinase/Akt pathway), as reported in young men with low birth weight characterized by increased future risk of insulin resistance and T2D. Using the LP model, Ozanne and colleagues studied the epigenetic regulation of the transcription factor hepatocyte nuclear factor 4-α (Hnf-4α) gene. This gene is the MODY (maturity-onset diabetes of the young) gene that has been the most extensively examined for association with common T2D and whose product is required for pancreatic β-cell differentiation and glucose homeostasis. They reported that maternal LP diet modified the interaction, at the Hnf4a locus, between the active P2 promoter and the enhancer region in pancreatic islets through alterations in histone marks. Precisely, LP islets were characterized by relative excess of the repressive mark H3K9me2 and loss of the active mark H3K4me1 at the enhancer region of the gene in pancreatic islets through alterations in histone marks. The hyperstimulated β cell: prelude to diabetes? [13]
reduction in Hnf4a expression. In addition, maternal diet amplified the age-associated epigenetic silencing of this locus. Since pancreatic islets from T2D patients have reduced HNF4A expression of a magnitude similar to that observed in LP islets and common variants at the HNF4A locus show association with T2D, this suggests that the epigenetic mechanisms observed may contribute to the development of pancreatic β-cell dysfunction and the subsequent development of T2D in humans (Susan Ozanne, Lecture).³

3. Role of microRNA in insulin secretion and action
MicroRNAs (miRNAs) are small noncoding RNA molecules that function in most cases as translational repressors: they exert their action by partially pairing with one or more sequences in the 3’ untranslated region of target mRNAs. They are ubiquitously expressed, but some of them are restricted to a limited number of tissues where they are involved in many physiological and pathological processes such as tissue differentiation, cell proliferation, apoptosis, and inflammation. Numerous miRNAs are highly expressed during pancreatic islet development (such as miR-7, miR-9, miR-375, and miR-376, for example, in human) and were shown to be involved in the control of adult β-cell mass and function (such as miR-9, miR-124a, miR-29a, and miR-33a). Recently, Regazzi’s group identified new miRNAs (miR-21, miR-34a, and miR-146a) with an induced expression by proinflammatory cytokines in β cells and in islets of prediabetic NOD (nonobese diabetic) mice, suggesting that they may be involved in defective insulin secretion and apoptosis observed in diabetes.⁶ Interestingly, they also characterized miR-29a/b/c as an important actor in impairment of glucose-induced insulin secretion (GIIS) and in β-cell apoptosis in type 1 diabetes (Romano Regazzi, Lecture).⁷

II- Impact of insulin resistance on β-cell function

1. Clinical Studies

a. Islet function in obese adolescents
T2D is affecting more and more adolescents. Excess adiposity is one of the major risk factors—along with puberty and ethnicity—for development of T2D in youth as these patients have a 10.4% prevalence of excess weight and a 79.4% prevalence of obesity. Data from autopsy showed a decrease in β-cell mass mainly because of an increase in β-cell apoptosis, but it is not completely clear whether this can be applied to all T2D patients and notably to adolescents especially because of the lack of safe and noninvasive methods to measure this parameter in vivo. However, it was reported that obese adult individuals with impaired fasting glucose had a roughly 50% deficit in β-cell fractional area compared with obese nondiabetics. Regulation of β-cell replication during infancy plays a major role in β-cell mass in adult humans. Indeed, β-cell mass expands by several fold from birth to adulthood, by growing in size rather than in number with a gradual decline thereafter to adulthood. Thus, β-cell function in adulthood strongly depends on β-cell development during childhood.Recently, Caprio’s group studied the progression of glucose tolerance in obese adolescents with normal glucose tolerance (NGT) and in those with impaired glucose tolerance (IGT) over two years. Obese adolescents with IGT are characterized by a progressive loss of β-cell glucose sensitivity during the first phase of insulin secretion, while obese T2D youth have both first and second phases impaired, accompanied by alterations in proinsulin-to-insulin processing. They showed that the adolescents with the highest 2-h glucose values during a hyperinsulinemic clamp procedure in the NGT group had similar changes to the adolescents of the IGT group, characterized
by decreased insulin sensitivity and first-phase insulin secretion. This suggests that β-cell function and insulin sensitivity are impaired even in youth classified as NGT and reflects a short transition time between NGT, IGT, and diabetes, illustrating that the progression of the disease is rather fast, unlike what is usually described in adults (Sonia Caprio, Lecture).

b. The role of genetics
As mentioned, many factors can explain the development of β-cell failure observed in T2D and among them the genetic component has a main role. Identification of relevant susceptibility genes has been difficult, mainly because the diabetes risk has been attributed to the interaction of multiple variant genes with the environment, where each of these genes only makes a small contribution to overall heritability. Studies reported that both insulin sensitivity (30%-40%) and the insulin response (38%) are heritable, and the disposition index (a quantitative measure that describes the relationship between β-cell sensitivity and insulin sensitivity) is heritable to a greater extent (67%). With the continual development of the genome-wide association study (GWAS), approximately 40 genes with single nucleotide polymorphisms (SNPs) associated with T2D have been identified (Figure 3).

2. Experimental Studies
a. Effect of diet on islet gene expression in mice
As introduced in chapter I2, Metabolic programming of insulin secretion and action, parental diets, in particular maternal protein restriction and paternal high-fat diet (HFD) can lead to a reprogramming of gene expression in the islets of the offspring, implicating an important role of epigenetic control in islet function. HFD is the most common intervention in experimental animal models for the study of obesity and T2D. However, many mouse strains show a genetic diversity that is comparable to that of the human population and thus differ widely in their physiological response to HFD as well as in their development of obesity, their insulin sensitivity, insulin secretion, and susceptibility for diabetes-related traits. For example, expression of either the leptin gene (ob/ob) or the leptin receptor gene (db/db) mutation on the C57BL/6 back-
ground resulted in a phenotype of massive obesity accompanied by insulin resistance with only transient diabetes, while the same mutations produced initial obesity and insulin resistance followed by life-shortening diabetes when present in the C57BL/KsJ strain.

- **C57BL/6J mice** are usually used for studies on diet-induced obesity and diabetes, although they have a high propensity to increase β-cell proliferation in response to insulin resistance. Interestingly, C57BL/6J mice fed a 16-week HFD are characterized by a 2-fold decreased expression of glutathione peroxidase 1 (Gpx1), a protein involved in the antioxidant defenses of β cells. This suggests that down-regulation of Gpx1 in pancreatic islets in response to a diabetogenic HFD may constitute an important factor contributing to the pathogenesis and progression of the disease. Although no significant associations were found with genetic variants of **GPX1** in the recent large-scale GWAS in diabetes, several studies have reported genetic association of **GPX1** variants with diabetes-associated complications.

- **New Zealand obese (NZO) mice** develop a polygenic disease pattern of obesity, hyperglycemia, hyperinsulinemia, hypercholesterolemia, and hypertension which in many ways resembles the human metabolic syndrome, and the prevalence for T2D is greatly increased in animals receiving a HFD. However, animals fed with a carbohydrate-free high-fat diet (CHFD) are protected from developing diabetes. Using laser capture microdissection and genome-wide transcriptome analyses, numerous transcripts involved in growth and development, protein processing and secretion, metabolism, and signaling were shown to be differentially regulated between HF and CHFD. Oxidative phosphorylation (OXPHOS) is the predominant gene set that was significantly upregulated in response to a HFD, demonstrating that a HF or high-carbohydrate diet enhanced islet oxidative metabolism. As a consequence, there was increased reactive oxygen species (ROS) production, including superoxide anions, hy-

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**Figure 3.** Schematic diagram of the pancreatic β cell showing the proposed subcellular localization of proteins encoded by diabetes-associated genes (from reference 9: Florez, Diabetologia. 2008;51(7):1100-1110. © 2008, Springer-Verlag). GCK encodes glucokinase, the glucose sensor of the β cell. KCNJ1 encodes the ATP-sensitive potassium channel Kir6.2, which interacts with the sulfonylurea receptor (SUR1, encoded by ABCC8) to regulate potassium currents across the cell membrane. HNF4A, TCF1 (encoding HNF1α), TCF2 (encoding HNF1β), HHEX, and TCF7L2 encode transcription factors produced in the β cell and implicated in pancreatic development. WFS1 encodes wolframin, a protein that regulates calcium transport in the endoplasmic reticulum. SLC30A8 encodes the ZnT-8 transporter responsible for transporting zinc into insulin secretion granules. CDKAL1 and CDKN2A/B are involved in the cyclin-dependent kinase pathway, and may thus influence β-cell regeneration. IGF2BP2 encodes a protein that binds IGF2 mRNA and directs it to specific subcellular locations for protein synthesis. It should be noted that many of these genes are also expressed in several other human tissues.
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drogen peroxide, and hydroxyl radicals, which led to increased expression of regulators of the cellular redox state, including catalase (CAT), Gpx1, peroxiredoxins, and thioredoxin-interacting protein, strongly implicating ROS and increased oxidative stress in the early state of β-cell failure (Hadi Al-Hasani, Lecture).

b. Identification of a novel diabetes susceptibility gene
To identify a diabetes susceptibility quantitative trait loci (QTL), Leibel and colleagues studied the F2 progeny of the intercross of obese Lepob/ob, diabetes-resistant C57BL/6J and diabetes-prone DBA/2J mouse strains. They cloned a candidate gene accounting for the QTL, that was designated “Lisch-like” (Li), encoding multiple tissue-specific transcripts in brain, liver, and islets, and predicted to encode a transmembrane protein that could mediate cholesterol transport and/or convey signals related to cell division. Using mice with reduced Li expression, they showed that Lisch-like is novel in structure among diabetes susceptibility genes, as it appears to alter β-cell development and glucose metabolism. Interestingly, the human ortholog, C1orf32, is in the middle of a 30-Mb region of Chr1q23-25 that has been repeatedly associated with T2D (Rudolph Leibel, Lecture).

c. IAPP and β-cell dysfunction
Low-grade systemic and localized islet inflammation have been suggested to contribute as causative factors in the development of T2D. One actor of inflammation involved in decreased β-cell mass is islet amyloid deposits that contain as their unique component the β-cell peptide islet amyloid polypeptide (IAPP, or amylin). IAPP is released from β cells in response to glucose and other stimuli that also trigger insulin secretion. Small aggregates or fibrils formed from amyloidogenic human IAPP (hIAPP) are toxic to β cells in culture and increase β-cell apoptosis and decrease β-cell mass in hIAPP transgenic mice. Moreover, hIAPP was recently shown to induce islet chemokine secretion (mainly interleukin 1[IL-1]), which promotes macrophage recruitment and activation leading to islet inflammation. Islet transplantation is a promising treatment for diabetes, but long-term success is limited by progressive graft loss and many studies raise the possibility that rapid amyloid formation in transplanted islets may be detrimental to graft function and mass, thus contributing to islet graft failure. Xenotransplantation of pancreatic islets, using pigs or other animals as islet donors, has received increasing interest in recent years, given the limited number of human islets available for clinical transplantation; interestingly, porcine islets have demonstrated long-term graft survival. Verchere’s group reported that the survival of transplanted porcine islets could be due, at least in part, to the fact that the porcine IAPP sequence differs from the human sequence at 10 positions and includes substitutions predicted to reduce its amyloidogenicity. These data suggest that islet amyloid–induced inflammation contributes to β-cell dysfunction observed in T2D and after islet transplantation (Bruce Verchere, Lecture).

d. Signaling from insulin resistant muscle to the β cell
T2D and obesity are characterized by dramatically increased circulating levels of tumor necrosis factor α (TNF-α). Although there is little evidence for elevated TNF-α in the skeletal muscle of individuals with T2D, its contribution toward skeletal muscle insulin resistance is well established. TNF-α is also believed to be a major cytokine involved in “conversation” between adipose tissue and muscle. In both rat and human primary β cells, the secretome from normally-insulin-sensitive muscle cells was reported to increase rat primary β-cell proliferation as well as GIIS. On the contrary, these parameters were decreased by incubation with conditioned medium from insulin resistant, TNF-α-treated skeletal muscle cells, while β-cell apoptosis was increased. This suggests that the insulin-resistant human skeletal muscle secretes...
myokines in response to TNF-α that impact negatively on β-cell proliferation and survival. Silencing of the mitogen-activated protein 4 kinase 4 (MAP4K4) gene prevents these β-cell impairments. These data reveal a possible new route of communication between skeletal muscle and β cells that may contribute to maintenance of β-cell functional mass in healthy subjects, as well as to the decrease seen in T2D (Karim Bouzakri, Lecture).18

III- Intrinsic hyperstimulation of β cells

1. Persistent hyperinsulinemnic hypoglycemia of infancy: a model for β-cell proliferation and survival

Hyperinsulinism of infancy (HI), also known as persistent hyperinsulinemic hypoglycemia (PHHI) of infancy, is a rare genetic disorder that occurs in approximately 1 in 50,000 live births. Most cases are caused by mutations in the subunits of the β-cell ATP-sensitive potassium channel (KATP channel), a minority of patients have glucokinase (GCK) or glutamate dehydrogenase mutations, and in 40%-50% of the patients, the genetic cause of the disease is still not known. The histologic appearance of the pancreata from affected children can be subdivided into 2 major forms: diffuse HI and focal HI. The diffuse HI is more common and bears some characteristics of neosodioblastosis such as the persistence of the neonatal-type β-cell distribution in older patients. The focal HI is generally easily recognized as a discrete region of adenomatous hyperplasia, whereas the rest of the pancreas appears normal for its age. Patients with genetic evidence of diffuse or focal HI who do not undergo surgery appear to enter clinical remission over a period of months to years. Whereas patients with diffuse HI progress to diabetes, patients with suspected focal HI glucose and insulin dynamics normalize. Glaser’s group studied the age-specific changes in human β-cell proliferation and apoptosis leading to the pancreatic remodeling normally seen in the postnatal period and compared them with pancreata from children with HI. They showed persistent increased β-cell proliferation and apoptosis in the HI pancreata, probably explaining the fetal-type β-cell distribution found in older patients with diffuse HI. The slow progressive decrease in insulin secretion observed clinically in these patients also suggested that the net rate of apoptosis was greater than that of proliferation leading to loss of β-cell mass (Benjamin Glaser, Lecture).19

2. Role of KATP channels

In the β cell, glucose metabolism turns on two distinct complementary sequences of events known as the triggering pathway and the metabolic amplifying pathway. The triggering pathway begins with the raising of the ATP/ADP ratio, which closes KATP channels, depolarizes the cell membrane, activates voltage-gated calcium channels (Ca2+ channels), and results in calcium influx, which in turn triggers exocytosis of insulin-containing granules (Figure 4).20 The metabolic amplifying pathway does not directly implicate KATP channels or any further rise in cytosolic global or subplasmalemmal [Ca2+]C, but augments the secretory response to the triggering Ca2+ signal by as yet unresolved mechanisms. Pancreatic KATP channels are hetero-octameric complexes of two separate proteins: the sulfonylurea receptor 1 (SUR1, ABCC8) subunit and the potassium channel Kir6.2 (KCNJ11) subunit. Antidiabetic agents that target KATP channels are a part of the treatment recommended by the American Diabetes Association and the European Association for the Study of Diabetes for T2D. Sulfonylureas (SUs), such as tolbutamide, gliclazide, and glibenclamide, inhibit KATP channel activity, causing membrane depolarization and triggering insulin secretion. Thus, SUs can uncouple metabolism from electrical activity and are widely used to treat T2D. However, many data have reported beneficial (eg, stimulation of β-cell pro-
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Liferation and protection against β-cell death) as well as deleterious (e.g., reduction in insulin content, increase in β-cell apoptosis, and reduction in GIR) effects of SUs. Conversely, K<sub>ATP</sub>-channel openers (KCOs), such as diazoxide, activate K<sub>ATP</sub> channels, thereby electrically silencing the cell at elevated glucose concentrations and inhibiting insulin secretion. According to the current concept, protection against β-cell death is attributed to KCOs instead of channel blockers as they improve insulin secretion when applied during continuous hyperglycemia and can help in the restoration of cellular insulin content.

**a. K<sub>ATP</sub> channels, hyperstimulation and hyperexcitation of β cells**

Under physiological conditions, glucose stimulation of β-cell electrical excitation leads to insulin secretion. Islets from mice with loss-of-function mutations in K<sub>ATP</sub> channels, such as Kir6.2<sup>−/−</sup> and Sur1<sup>−/−</sup> mice, are continuously hyperexcited even at low glucose concentrations that normally do not lead to hyperstimulation of insulin secretion. Neonatal mutant mice exhibit transient hyperinsulinemia and hypoglycemia, but islets from adults show a dramatic loss of insulin secretion at all glucose concentrations, and the animals are relatively hypoinsulinemic. In Kir6.2<sup>[Gly132Ser]</sup> mice, which specifically lack β-cell K<sub>ATP</sub> channels, loss of β-cell mass was reported, although hyperglycemia was apparently spontaneously improved and insulin content even increased in older mice. Conversely, β-cell–specific Kir6.2<sup>[AAA]</sup> dominant-negative mice, which lose K<sub>ATP</sub> channel activity in only about 70% of β cells, exhibit elevated circulating insulin levels that persists through adulthood, with essentially normal insulin content and islet morphology. Heterozygous Kir6.2<sup>[Gly132Ser]</sup> and Sur1<sup>−/−</sup> mice, with about 60% reduction in K<sub>ATP</sub> density in every cell, show a similar phenotype. Complete lack of K<sub>ATP</sub> activity has been reported in β cells from some HI patients, but the phenotype of many HI mutations would suggest that K<sub>ATP</sub> is not always completely absent.

On the contrary, gain-of-function mutations in mice expressing mutant K<sub>ATP</sub> channels with reduced ATP sensitivity—either because of reduced ATP affinity (mutations in the ATP binding site) or through allosteric enhancement of open probability—only in pancreatic β cells (Kir6.2<sup>[ΔN2-30]</sup>) developed severe neonatal diabetes mellitus (NDM) and died. Indeed, K<sub>ATP</sub> mutations that cause loss of ATP sensitivity are expected to maintain the membrane in a hyperpolarized state with hyposecretion of insulin. To overcome the drastic effect of the mutation, Nichols’ group used the inducible model strategy for the Rosa26-Kir6.2<sup>[K185Q, ΔN30]</sup> transgene and observed that the resulting significant loss of ATP sensitivity led to strong glucose intolerance that progressed to severe diabetes. Growth retardation and dramatic reduction in insulin content, accompanied by profound loss of β-cell mass over time were also noticed as secondary effects. Importantly, syngeneic islet transplantation under the kidney capsule or chronic treatment with glibenclamide two days prior to onset of transgene expression induction prevented the development of diabetes and also maintained both pancreatic islet architecture and β-cell mass. This result clearly showed that secondary loss of insulin content and secretion was a consequence of systemic diabetes. Interestingly, glibenclamide was ineffectual once the disease had developed and insulin content was lost. One can suppose that exposure to episodic hyperglycemia may lead to similar secondary progression in human NDM patients, explaining that SUs can effectively trigger insulin secretion, but requirements tend to increase with length of disease and in some cases, SU therapy becomes ineffective (Colin Nichols, Lecture).

**b. K<sub>ATP</sub> channels and oxidative stress**

Glucolipotoxicity caused by chronic hyperglycemia and an excess of lipids is a main factor involved in the development of T2D. In fact, β cells often survive for a long period of time with exposure to high glucose and free fatty acid (FFA) concentrations that contribute to the slowly progressing impairment of β-cell function, including reduced β-cell mass and loss
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of insulin content. Numerous studies have reported that glucolipotoxicity might be related to oxidative stress which refers to a persistent imbalance between excessive ROS production and limited antioxidant defense, a situation that occurs in β cells during pathogenesis of diabetes. Briefly, the mitochondrial respiratory chain is a major source of ROS in β cells. The superoxide anion (O$_2^-$) is tightly coupled to mitochondrial metabolism and is generated by a single electron reduction of molecular oxygen at the inner mitochondrial membrane mainly by complexes I and III. O$_2^-$ is a reactive molecule that is converted to less active hydrogen peroxide (H$_2$O$_2$) by superoxide dismutase (SOD) isoenzymes. H$_2$O$_2$ is further detoxified by CAT and GPxs. A growing amount of evidence indicates that ROS are involved in the maintenance of normal β-cell glucose responsiveness, as well as in the impairment of secretory capacity and cell viability, both parameters contributing to β-cell failure.$^{22}$ This suggests that ROS may have different actions depending on whether cellular concentrations are either below or above a specific threshold, i.e., signaling versus toxic effects. Rodent β cells are highly sensitive to oxidative stress because their antioxidant defenses are very low. Overexpression of antioxidant enzymes or antioxidant treatment protects β cells against the effects of nitric oxide (NO) and oxygen radicals and has beneficial effects on β-cell mass and insulin content in diabetic mice. Human β cells seem to be less prone to oxidative stress, possibly because they have greater CAT and SOD activity. Prediabetic and newly diagnosed T2D patients have increased oxidative stress and decreased antioxidant defense systems. Drews’ group showed that H$_2$O$_2$ can decrease the ATP/ADP ratio leading to the opening of β-cell K$_{ATP}$ channels, hyperpolarization of the plasma membrane potential, and impaired insulin release. Interestingly, direct inhibition of K$_{ATP}$ channels with the SU tolbutamide restored GII, suggesting that H$_2$O$_2$ interferes with β-cell metabolism whereas the secretory machinery of the cell remains intact. Importantly, Surt1$^{+/+}$ mice turned out to be less sensitive to ROS-induced inhibition of insulin secretion in vitro and streptozotocin (STZ)-induced diabetes in vivo. These mice showed an approximately 2-fold upregulation in SOD, CAT, and GPx activity that drastically reduced H$_2$O$_2$ - and NO-induced apoptotic cell death compared with Surt1$^{-/-}$ mice. Similarly, gliazide and tolbutamide treatment led to an increase in antioxidant enzyme activity, probably through Ca$^{2+}$-dependent upregulation and attenuated ROS-induced apoptosis.$^{22}$ Nevertheless, it is noteworthy that a global increase in apoptosis has been described for tolbutamide whereas gliazide has no proapoptotic potency. This difference can be explained by the free radical–scavenging property of gliazide.

**Figure 5** sums up the possible approaches to combat oxidative stress: reduction in ROS formation, upregulation of antioxidant enzymes, and application of antioxidants (Gisela Drews, Lecture).$^{22}$

**2. β-Cell fate in defective cellular calcium regulation**

The fundamental second messenger for insulin release is Ca$^{2+}$ (Figure 4).$^{19}$ Indeed, a number of nutrients, hormones, and pharmacological substances that influence in-

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**Figure 4.** Schematic illustration of the glucose-stimulated insulin secretory pathway in β cells (from reference 20: Remedi and Nichols. Cell Metab. 2009;10(6):442-453. © 2009, Elsevier Inc.). (A) Hematoxylin-eosin stained paraffin section of mouse pancreas. The pancreas is composed of exocrine tissue and endocrine tissue (islet of Langerhans). Islets contain different cell types, including the insulin-secreting β cells. Arrows point to exocrine and endocrine tissue. (B) Schematic illustration of the β-cell glucose-stimulated insulin secretion pathway. Glucose entering the β cell through glucose transporters (GLUT2) is phosphorylated by glucokinase and metabolized by glycolysis (cytoplasm) and tricarboxylic acid (TCA) cycle (mitochondria). A rise in the [ATP]/[ADP] ratio resulting from oxidative metabolism inhibits the ATP-sensitive K$^{+}$ channels (K$_{ATP}$) at the cell surface, causing membrane depolarization and opening of voltage-dependent Ca$^{2+}$ channels (VDCC). This results in a rise in intracellular Ca$^{2+}$ which stimulates insulin secretion. Voltage-dependent outward K$^{+}$ channels (Ko) are involved in membrane repolarization and cessation of insulin secretion. Factors that impair ATP production or downstream signaling are expected to suppress glucose-stimulated insulin secretion (GIIS). Several genes that directly or indirectly alter ATP production and therefore underlie a diabetic or hyperinsulinemic phenotype when mutated (humans and/or mouse models) are shown in color: glucokinase (GK), nicotinamide nucleotide transhydrogenase (NNT), uncoupling protein 2 (UCP2), mitochondrial DNA mutations (mtDNA), and glutamate dehydrogenase (GDH). Mutations that may indirectly affect channel activity are also shown in color. (C) Schematic illustration of the electrical activity of β cells and the temporal response to elevated glucose. In low (3 mM) glucose, the membrane is hyperpolarized due to K$_{ATP}$ activity, intracellular Ca$^{2+}$ ([Ca$^{2+}$]) is low and insulin is not secreted. When glucose is elevated to stimulatory levels (11 mM), K$_{ATP}$ channels close, and the membrane depolarizes due to L-type VDCC activity. Bursts of action potentials (AP), involving both VDCC and Kv channels, result in slowly elevated [Ca$^{2+}$], which in turn triggers insulin secretion.
insulin secretion alter Ca\(^{2+}\) uptake and/or Ca\(^{2+}\) influx. Whereas millimolar concentrations of Ca\(^{2+}\) are present in the extracellular space and equally high amounts of bound Ca\(^{2+}\) may be found in intracellular stores, the intracellular free Ca\(^{2+}\) concentration in the β-cell cytoplasm is kept below 100 nM under unstimulated conditions. This is achieved by various types of Ca\(^{2+}\)-ATPases that pump out cytoplasmic Ca\(^{2+}\) and by Ca\(^{2+}\)-buffering actions of many cytoplasmic Ca\(^{2+}\)-binding proteins. When the β cell is stimulated, cytosolic Ca\(^{2+}\) increases as a result of either Ca\(^{2+}\) influx from the extracellular space or Ca\(^{2+}\) release from intracellular stores.

The Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) is an antiporter membrane protein that removes calcium from cells. This electrogenic transporter uses the energy that is stored in the electrochemical gradient of Na\(^{+}\) by allowing Na\(^{+}\) to flow down its gradient across the plasma membrane in exchange for the countertransport of Ca\(^{2+}\), with a stoichiometry of 3 Na\(^{+}\) for 1 Ca\(^{2+}\). It extrudes Ca\(^{2+}\) in parallel with the plasma membrane’s ATP-driven Ca\(^{2+}\) pump and as a reversible transporter, it also mediates Ca\(^{2+}\) entry in parallel with various ion channels.

Four isoforms of the NCX (NCX1 to NCX4) have been cloned and the β cell expresses various NCX1 splice variants in a species-specific pattern (NCX1.3 and 1.7 in the rat; NCX1.2, 1.3, and 1.7 in the mouse), in variable and different proportions. Interestingly, overexpression of the exchanger led to the depletion of endoplasmic reticulum (ER) Ca\(^{2+}\) stores causing ER stress, reduction of β-cell growth, and finally, activation of β-cell death by apoptosis.\(^2^4\) On the contrary, Ncx1 heterozygous deficient mice (Ncx1\(^{+/−}\)), with NCX1 downregulation, were reported to have an increased GIIS and an enhancement of both phases of insulin release, but no increased mitochondrial glucose metabolism. Insulin content was doubled in Ncx1\(^{+/−}\) mice compared with Ncx1\(^{+/+}\) mice, accompanied by an enhancement in proinsulin staining. In adult mice, β-cell mass had risen 100% in Ncx1\(^{+/−}\) mice compared with Ncx1\(^{+/+}\) mice; this was mainly due to β-cell proliferation resulting from the activation of the cal-

**Figure 5.** Possible targets for β-cell protection against oxidative stress (from reference 22: Drews et al. Pflugers Arch. 2010;460(4):703-718. © 2010, Springer-Verlag). Schematic drawing of stimulus-secretion coupling of a β cell showing the sites of reactive oxygen species (ROS) production and possible targets to reduce oxidative stress.
cineurin/nuclear factor of the activated T-cell signaling pathway by increased cellular Ca\(^{2+}\). Finally, \(\text{Ncx1}^{+/−}\) islets were protected against hypoxia. Thus, the effectiveness of \(\text{Ncx1}^{+/−}\) islet transplantation was tested, because in clinical islet transplantation, up to 70% of the transplanted β-cell mass is destroyed in the early post-transplant period mainly due to prolonged hypoxia during the revascularization process. Interestingly, transplantation with \(\text{Ncx1}^{+/−}\) islets was at least twice as efficient as with \(\text{Ncx1}^{+/+}\) islets. Data both on improvement of insulin production and islet transplantation imply that \(\text{Ncx1}\) is a potential new therapeutic target (André Herchuelz, Lecture).

4. β-Cell fate in mice with activating GCK mutations

GCK is the high-Km enzyme that phosphorylates glucose on its entry into the β cell (Figure 4). Persistent hyperinsulinemic hypoglycemia (PHH) due to a novel GCK mutation was recently described. The mutation led to a markedly increased affinity for glucose and to the subsequent increase in intracellular glucose flux and lower threshold for GIIS. Moreover, abnormally large pancreatic islets were reported that showed both proliferating and apoptotic β cells, probably resulting from increased intracellular glucose flux. This data in human was consistent with previous findings in murine models. In contrast, adult mice with tamoxifen-inducible deletion of GCK specifically in β cells developed severe hyperglycemia and hypoinsulinemia, consistent with the inability of mutant β cells to sense glucose and secrete insulin. Mutant mice also showed a dramatic drop in β-cell proliferation and increased β-cell apoptosis resulting in a 2-fold reduction in total β-cell mass 2 months after GCK deletion. As expected, increasing GCK activity stimulated the β-cell proliferation rate in a K\(_{ATP}\) channel/membrane depolarization–dependent way. This work suggests a role for GCK and glucose metabolism in the regulation of β-cell secretion and replication, such as a short pulse of glucose metabolism, as after a meal, would trigger β-cell secretion, while more persistent activation of the pathway would trigger β-cell replication. This implies that GCK activators could have beneficial effects on β-cell mass and on the contrary, that normalization of blood glucose in diabetic patients could lead to the suppression of the glucose metabolism–induced mitogenic effects on β cells (Yuval Dor, Lecture).

IV- Modulation of β-cell function by secretory products

1. Autoregulation of insulin secretion

Insulin release from β cells is directly controlled by the blood glucose level and modulated by the autonomous nervous system and autocrine and paracrine regulations. The latter is facilitated by the architecture of human islets, with non–β cells distributed throughout the islet, rather than confined to the islet periphery as in rodents.

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter contained in insulin secretory granules. It is thus released from β cells upon glucose stimulation by Ca\(^{2+}\)-dependent exocytosis, but also via a nonvesicular and glucose-independent pathway. GABA stimulates insulin secretion and inhibits glucagon secretion in rodent islets by activating ligand-gated Cl– channel GABA\(_A\) receptors (GABA\(_A\)R) in α cells. However, through G-protein coupled GABA\(_B\) receptors (GABA\(_B\)R), GABA inhibits GIIS in rodents and human. Braun and colleagues showed that in human islets, GABA stimulates β-cell insulin secretion by activating GABA\(_B\)R and that glucose stimulates vesicular release of GABA from β cells. This suggests that signaling via GABA and GABA\(_B\)R stimulates insulin secretion by a positive autocrine feedback loop in human β cells. Moreover, the presence of GABA\(_B\)R in non–β cells suggests that GABA may also be involved in the regulation of somatostatin and glucagon secretion.
ATP is released by β cells upon glucose stimulation and Ca\(^{2+}\)-dependent exocytosis, with IAPP and serotonin. Interestingly, through its binding on P2X3 receptor, a ligand-gated, nonselective cation-conducting channel, extracellular ATP was shown to stimulate membrane depolarization and GIIS in human islets in a positive feedback pathway.\(^{29}\)

Zinc is co-released with insulin during exocytosis and was reported as a paracrine inhibitor of glucagon secretion by α cells through a K\(_{\text{ATP}}\) channel–dependent mechanism. Polymorphisms of the zinc efflux transporter ZnT8 gene were shown to increase the risk of T2D in human. Moreover, mice with a β-cell–specific ZnT8 deletion are glucose intolerant and have reduced GIIS and insulin-processing enzyme transcripts and increased proinsulin levels, suggesting that ZnT-8 is absolutely essential for proper β-cell function. In contrast, mice with α-cell–specific ZnT8 deletion show no evident abnormalities in plasma glucagon and glucose homeostasis (Matthias Braun, Lecture).\(^{30}\)

2. Insulin positive feedback on β-cell function and effects of intensified insulin treatment in T2D

The role of insulin in β-cell insulin secretion in human was long unclear because of the difficulty to distinguish between the role of endogenous and exogenous infused insulin. However, an isoglycemic-hyperinsulinemic clamp procedure using B28-Asp-insulin (which can be distinguished immunologically from endogenous insulin) was recently used to show that insulin does enhance GIIS in healthy humans. Indeed, pre-exposure to exogenous insulin increased the C-peptide response and the endogenous insulin secretory response to glucose by approximately 40%.\(^{31}\) This effect of insulin was reported to be independent of FFA concentrations in healthy humans\(^{32}\) and was attenuated in insulin-resistant subjects. This is consistent with an effect of insulin to regulate β-cell function in humans in vivo and has glucose-lowering therapeutic implications (Allison Goldfine, Lecture).\(^{33}\) Indeed, antidiabetic medication for T2D treatment acts to prevent the deleterious effects of hyperglycemia, but the medication’s ability to preserve β-cell function may be insufficient,\(^{34}\) and insulin therapy is often ultimately prescribed. However, the use of short-term intensive insulin therapy (IIT) early in the course of T2D—when sufficient β-cell mass remains to enable functional improvement—has emerged as a therapeutic option that may offer favorable long-term effects on β-cell function. IIT is defined as delivering frequent or continuous doses of insulin that are intended to achieve tight glycemic control, which is currently defined as blood glucose levels no more than 150 mg/dL. IIT was shown to induce euglycemia in subjects with newly-diagnosed diabetes, which was maintained during 2 years or more in some patients. Indeed, IIT was shown to partially restore first-phase insulin secretion and improve scores for the Homeostasis Model Assessment of β-cell function (HOMA-B) and the fasting proinsulin/insulin ratio. Interestingly, IIT resulted in significant improvement in quality of life and treatment satisfaction, demonstrating patient acceptability of early insulin therapy (Bernard Zinman, Lecture).\(^{35}\)

V- Conclusion

T2D develops in response to both overnutrition and lack of physical activity in subjects that are predisposed to both insulin resistance (and/or hyperinsulinemia) and β-cell dysfunction. The overworked-β-cell hypothesis proposes that lowered glucose-potentiarted insulin secretory responses in diabetes are secondary to hyperstimulated insulin secretion and depletion of the β-cell insulin stores. Recent evidence shows that β-cell dysfunction appears early, long before the onset of prediabetes, when glycemia is still
classified as NGT. This evolution from NGT to IGT and finally to established T2D in adult patients is accelerated in obese adolescents. This progressive failure leads to poorly functioning, dedifferentiated β cells and loss of β-cell mass from apoptosis. Research effort needs to focus on the factors that make islets susceptible to dysfunction and failure, particularly those that are acquired in early life, as these may be preventable. Even after diagnosis of T2D, β-cell function continues to worsen such that subjects progress from needing changes in diet/exercise only to requiring oral hypoglycemic agents and eventually insulin for achievement of adequate glycemic control. Future therapies will be directed not only toward achievement of euglycemia, but also alteration of the course of the disease by reversing the processes of β-cell failure.
Lectures during IGIS meeting

- Hadi Al-Hasani  Effect of diet on islet gene expression in mice
- Karim Bouzakri  Signaling from insulin resistant muscle to the β cell
- Matthias Braun  Autoregulation of insulin secretion in human islets
- Sonia Caprio  Islet function in obese adolescents
- Yuval Dor  β-cell fate in mice with activating glucokinase mutations
- Gisela Drews  Role of KATP channels in β-cell resistance to insult
- Gary Felsenfeld  Epigenetic control of insulin gene transcription
- Benjamin Glaser  Persistent hyperinsulinemic hypoglycemia of infancy: a model for β-cell proliferation and survival…
- Allison Goldfine  Insulin positive feedback on β-cell function
- Hans-Ulrich Häring  Analysis of β-cell function: impact of incretin resistance and insulin resistance
- André Herchuelz  β-cell fate in defective cellular calcium regulation
- Steven E. Kahn  Interactions between genetic background, insulin resistance and β-cell function
- Rudolph L. Leibel  Lisch-like, a modifier of β-cell function that causes islet hyperactivity in db/db mice.
- Colin Nichols  The diabetic mouse β-cell: hyperstimulated may not be hyperexcited
- Susan E. Ozanne  Metabolic programming of insulin action and secretion
- Romano Regazzi  MiRNAs in insulin action and secretion
- Bruce Verchere  IAPP and β-cell dysfunction
- Bernard Zinman  Long-term effects of short-term intensified insulin treatment in type 2 diabetes: β-cell rest?
References:


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