Review

# A direct look at the dysfunction and pathology of the β cells in human type 2 diabetes

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A B S T R A C T

β cells uniquely produce and secrete insulin under the control of several, integrated signals, to maintain blood glucose concentrations within a narrow physiological interval. β cell failure is key to the onset and progression of type 2 diabetes, due to impaired function and reduced mass. In this review we focus on several features of human β cell dysfunction and pathology in type 2 diabetes, as revealed by direct assessment of isolated islet traits and examination of pancreatic tissue from organ donors, surgical samples or autoptic specimens. Insulin secretion defects and pathology findings are discussed in relation to some of the major underlying mechanisms, to also provide clues for conceiving better prevention and treatment of type 2 diabetes by targeting the pancreatic β cells.

## 1. Introduction

β cells are unique endocrine cells that synthetize, store and secrete insulin under the control of multiple and integrated signals, thus tightly regulating blood glucose concentrations. They have a diameter of 10 μm on average, and each of them contains approximately 20 pg insulin [1–4]. β cells are the most represented cell type in pancreatic islets, comprising 50–80 % of all islet cells [1–4]. Hence, the content of insulin per islet has been calculated to be approximately 25 ng, with ample variations between studies [5–7]. In human adults the number of islets ranges 0.5–4 millions [8], and islet size may vary from 30 to 40 up to 400 − 500 μm in diameter [1–4]. Therefore, insulin content of the human pancreas spans widely, approximately from 60 to 200 μg/g, and is characterized by marked intersubject variability (up to ten-fold) [6,9,10]. Part of these variations may also be due to the fact that the published information is occasionally fragmentary, which sometimes makes the comparison of data from different groups challenging [7].

β cell failure is key to the onset and progression of any type of diabetes, due to the interplay of genetic and acquired factors, acting via several pathophysiological mechanisms [11–20]. In type 2 diabetes, the most common form of this heterogenous disease, β cells have peculiar dysfunctional and pathological features, that will be addressed in this article. Focus will be on human islets, with data mainly deriving from direct β cell functional assessments and histological evaluations. The presented evidences provide insights on several issues associated with β

cell incompetence in human type 2 diabetes, supporting the need of therapeutical strategie targeting the β cells to better prevent and treat this disease.

## 2. Insulin secretion from the normal β cells

In normal individuals, β cells release a total of 30–70 units of insulin per day (essentially depending on body weight, physical activity and nutritional habits), half of which under basal condition and the remaining in response to meals [21,22]. The most important regulator of insulin release is glucose, that acts as both a trigger as well as an amplifier of insulin secretion [21–25]. Several other physiological molecules regulate insulin secretion [5,21,22], including non-carbohydrate nutrients, hormones and neurotransmitters (Table 1).

Insulin secretion is a very dynamic process [21,22]. Intravenous administration of glucose elicits a biphasic β cell response, with an initial insulin burst lasting 5 − 8 min (first-phase), followed by a return towards pre-stimulation levels and then by a less acute and more prolonged insulin increase (second-phase). After oral glucose, insulin release reaches a peak after approximately 60 − 90 min and decrease to pre-load levels after 4 − 5 hours. Notably, insulin secretion in response to oral glucose is more pronounced than that observed after an intravenous infusion achieving the same glucose levels [21,22,26–28]. This greater response elicited by oral glucose is due to the action of intestinal hormones, released after nutrient ingestion, and is referred to

**Table 1**
Main physiological regulators of insulin release from β cells.

| *Nutrients* | *Action* |
|---|---|
| Glucose | Stimulation |
| Certain aminoacids (arginine, leucine + glutamine) | Stimulation |
| Certain fatty acids (in particular if > C12) | Stimulation |
| Ketones | Stimulation |
| | |
| *Hormones* | *Action* |
| Glucagon | Stimulation |
| GLP-1 | Stimulation |
| GIP | Stimulation |
| CCK | Stimulation |
| VIP | Stimulation |
| Gastrin | Stimulation |
| Secretin | Stimulation |
| Somatostatin | Inhibition |
| Ghrelin | Inhibition |
| | |
| *Neurotransmitters/Neuropeptides* | *Action* |
| Acethylcholine | Stimulation |
| Adrenaline (β2 receptors) | Stimulation |
| Adrenaline (α2 receptors) | Inhibition |
| Noradrenaline (α2 receptors) | Inhibition |
| NPY | Inhibition |

GLP-1, Glucagon-Like Peptide 1; GIP, Glucose-dependent Insulinotropic Peptide; CCK, Cholecystokinin; VIP, Vasoactive Intestinal Peptide; NPY, Neuropeptide Y.

as the "incretin effect" [26–28]. The two most important hormones involved in the incretin effect are GLP-1 (Glucagon-Like Peptide-1) and GIP (Glucose-dependent Insulinotropic Peptide) [26–28].

Neurotransmitters and neuropeptides also regulate insulin release [29,31]. Some of them can stimulate insulin secretion (such as acet-ylcholine and adrenaline via β2 receptors), or inhibit the release of the hormone (including adrenaline and noradrenaline via α2 receptors). Interestingly, similar to what neurons do, islet endocrine cells appear to be able to synthesize, accumulate and secrete a number of neuro-transmitters, that could therefore modulate β cell function by paracrine interactions [30,31].

In addition, insulin release in normal subjects is pulsatile [32–34]. Two major frequencies characterize the pulses. Ultradian oscillations have a period of approximately 2 h, and probably result from a feed-back loop between glucose production and insulin secretion. Faster oscillations were initially reported to have a period of ∼ 15 min in peripheral blood, and then more accurately shown to occur with a periodicity of 4 − 6 min. This high frequency pulses are likely due to a pacemaker function in the islets themselves. Of interest, glucose in-creases the amplitude of the oscillations, but not their frequency [33,34].

Several of the β cells functional features studied *in vivo* have been more directly investigated with islets isolated from the pancreas of multiorgan donors [35–37]. Earlier assessments [35,38,39] demon-strated that perifused human islets were responsive to glucose stimu-lation, as shown by several-fold increase of insulin release at higher glucose concentration, followed by a prompt return to baseline values upon cessation of the stimulus. These findings have been then con-firmed by numerous other studies evaluating glucose stimulated insulin release under static incubation or dynamic conditions [40–45]. A few reports also show that human islets isolated from non-diabetic donors can respond to secretagogues others than glucose, such as certain aminoacids, fatty acids, hormones and drugs [41,46–50]. An elegant study has simultaneously examined most of these conditions by peri-fusion experiments [51], showing the concentration dependence of glucose stimulated insulin release (0–30 mmol/l), the induction of bi-phasic insulin secretion – with a prominent first phase - upon an in-crease from 1 to 15 mmol/l glucose level, the triggering effect of tol-butamide (a sulphonylurea agent), the rapid secretagogue action of the combination of glutamine with leucine (glutamine alone was not

effective), and the stimulatory properties of arginine.

In summary, normal β cells integrate multiple metabolic, hormonal and neuronal signals to release insulin in strictly regulated patterns, to maintain plasma glucose levels in the relatively narrow normal range. Although quantitative insulin secretion data of β cells in isolated human islets are sometimes inconsistent (reviewed in 7), available information shows that *ex vivo* islet functional features recapitulate most of the main qualitative and quantitative features of *in vivo* insulin release.

## 3. Insulin secretion from the β cells in type 2 diabetes

Type 2 diabetic subjects have blood insulin levels that are in-appropriately low for the prevailing glucose concentrations [6,11,21,52]. Abnormalities of the temporal pattern of insulin secretion in this form of diabetes have been shown as well, that include loss or blunted first phase release in response to intravenous glucose and al-terations of pulsatility cycles [6,11,21,52]. Some of these features have been investigated also *ex vivo* with islets isolated from type 2 diabetic donors [40,41,44,53–56]. In an early work islets were prepared from intraoperative biopsies and the results showed that the samples of type 2 diabetic subjects had a normal insulin content and a normal or even high biosynthesis, and that insulin release could be induced by glucose [53]. A few years later the same authors confirmed that isolated type 2 diabetic islets had a rate of insulin secretion under incubation with glucose similar to that of control cells [54]. However, all the studies available afterwards have found more or less profound alterations of β cell function in type 2 diabetic islets. In a report published in the early '90 s it was shown that glucose-stimulated insulin release as well as glucose oxidation/utilization were lower in diabetic than non-diabetic preparations [55]. However, the secretory response to a combination of L-leucine and L-glutamine appeared less markedly impaired [55]. When islets isolated from eight type 2 diabetic and nine non-diabetic donors were evaluated by perifusion experiments [40], it was observed that basal insulin secretion was superimposable between normal and dis-eased islets. However, β cells from diabetic donors released sig-nificantly less insulin in response to increasing glucose levels, and also showed a higher glucose threshold for the triggering of insulin release. In the same study, the functional incompetence of diabetic islets was also demonstrated by transplantation studies. The authors implanted an equivalent amount of non-diabetic and type 2 diabetic islets into dia-betic mice, and the diseased islets did not fully restore normoglycemia in comparison with normal cells [40]. The reduced β cell response to glucose of islets prepared from type 2 diabetic donors has been then confirmed in larger series [44]. In another study, insulin secretion was measured in response to glucose, glibenclamide and arginine in isolated non-diabetic and type 2 diabetic islets, under perifusion conditions [41]. Again, no significant difference in insulin release was observed at 3.3 mmol/l glucose. However, when challenged with 16.7 mmol/l glu-cose, diabetic islets secreted significantly less insulin than the non-diabetic cells. Interestingly, insulin secretion from the diabetic islets during glibenclamide and arginine stimulation was better preserved than that in response to glucose. In line with this, reduced glucose-stimulated insulin release and better maintained arginine-stimulated insulin secretion has been recently confirmed in a series of 61 non-diabetic and 19 type 2 diabetic islet preparations [56].

Notably, some evidence suggests that β cell dysfunction of type 2 diabetic islets can be rescued, at least in part, by direct exposure of isolated islets to certain pharmacological compounds. When islets iso-lated from 6 type 2 diabetic donors were exposed for 24 h to ther-apeutical concentration of the oral antidiabetic agent metformin, glu-cose-induced insulin release improved, the amount of insulin granules increased, and the expression of genes involved in oxidative stress (i.e. NADPH-oxidase, catalase, GHS-peroxidase and Heme oxygenase-1) decreased [57]. This latter result is of interest, since it has been re-ported that oxidative stress could be one the major mechanisms through which nutrient overload (increased free fatty acid and/or glucose

levels) leads to β cell impairment [16,18–20].

In another study, islets prepared from 11 non-diabetic and 7 type 2 diabetic organ donors matched for age and body mass index were exposed to exendin-4 [a dipeptidyl peptidase IV (DPP-IV)-resistant glucagon-like peptide-1 (GLP-1) mimetic] for 48 h and then studied functionally and molecularly [48]. It was found that, after treatment, glucose-stimulated insulin secretion increased of approximately 30 %, which was accompanied by higher expression of genes involved in β cell function and identity [48]. The beneficial effects of hormones of the incretin system have been confirmed afterwards [58]. Finally, a recent study has shown that exposure of diabetic islets to rapamycin was associated with improved insulin secretion and better preserved ultrastructure of insulin granules, mitochondria and the endoplasmic reticulum [59]. The favourable actions of rapamycin were likely due to the activation by this compound of macroautophagy, a process that eliminates damaged intracellular structures and has been found to be hampered in type 2 diabetic β cells [60].

Therefore, β cell dysfunction in type 2 diabetes can be directly assessed with islets from subjects with the disease, independently from the counfounding effects of the prevailing *in-vivo* metabolic status. More severe defects of insulin secretion in response to glucose than other secretagogues and possible rescue of β cell function are major features of type 2 diabetes pathophysiology.

## 4. Mechanisms of β cell insulin secretion dysfunction in type 2 diabetes

As mentioned above, glucose is the most important regulator of insulin release from the β cells, which are however responsive to several other triggering or amplifying molecules as well (Table 1). Direct assessment of β cell function by the use of isolated human islets has indicated that type 2 diabetic β cells show a more marked defect in the response to glucose than other secretagogues, such as certain pharmacological compounds (sulphonylureas) and aminoacids (arginine in particular) [41,55,56]. To elicit its effects on insulin release glucose has to be fully metabolized by the β cells to produce ATP and trigger the successive steps leading to the exocytosis of the insulin granules [5,61,62] (Fig. 1). However, sulphonylureas induce insulin secretion acting at a later step, by binding to the $K_{ATP}^{+}$ channels, determining their closure and causing membrane depolarization [63] (Fig. 1). As for arginine, it exerts its action by impacting on the distal exocytosis processes. This aminoacid enters the β cells through the CAT 2A cationic aminoacid transporter, thus causing excess of positive charges and subsequent depolarization of the plasma membrane [64,65] (Fig. 1). All this suggests that the type 2 diabetic β cells could have specific and more marked impairment of the mechanisms responsible of glucose metabolism.

The steps leading to glucose-stimulated insulin release have been reviewed in depth [61,62]. Glucose enters the cytoplasm through facilitating transporters, predominantly Glut1 in humans. Then the hexose undergoes phosphorylation in the glycolytic pathway by glucokinase (a high Km isoform of hexokinase, namely hexokinase IV). The successive glycolysis reactions lead to the formation of pyruvate, the three-carbon molecule that is the final product of the cascade. In normal β cells, the enzyme lactate dehydrogenase, that converts pyruvate to lactate along the anaerobic respiration, is normally expressed at very low level, and therefore pyruvate enters the mitochondrion to be oxidized in the aerobic respiration. Interestingly, gene expression of glucose transporters and glucokinase has been found to be lower in human type 2 diabetic islets than in control preparations [41,54,66,67] (Fig. 1). Furthermore, a study performed with β cells yielded by laser capture microdissection [68] indicated that aldolase B was highly expressed and upregulated in type 2 diabetic β cells. This finding has been then confirmed with isolated islets [56] and it has been shown that aldolase B is negatively associated with insulin secretion [56,69].

A key component involved in glucose metabolism is the

mitochondrion (Fig. 1). Once inside this organelle, the pyruvate produced by the glycolytic process is decarboxylated to form citrate, a metabolite of the tricarboxylic acid (TCA) or Krebs cycle. The activation of the TCA cycle leads to the production of the reducing equivalents NADH and FADH2, which are used for the generation of ATP in the electron transport chain, which is then translocated to the cytosol by the adenine nucleotide translocator. Several evidences do currently indicate that mitochondria could play a major role in the dysfunction of type 2 diabetes β cells [25,70,71] (Fig. 1). β cell mitochondria in type 2 diabetes look round shaped and with reduced electron density (Fig. 2a). In addition, when adenine nucleotide content was measured, it was found that diabetic β cells were not able to increase their ATP content following acute glucose stimulation (Fig. 2b) [70]. Therefore, the ATP/ADP ratio was almost 50 % lower in type 2 diabetic than control islets, which, accordingly, was associated with altered hyperpolarization of the mitochondrial membrane, and augmented expression of UCP-2, complex I, and complex V of the respiratory chain were observed [70]. In addition, the activities of the mitochondrial enzymes glycerol phosphate dehydrogenase, pyruvate carboxylase and succinyl-CoA:3-ketoacid-CoA transferase have been reported to be markedly lower in the diabetic compared with control islets [55,72]. Furthermore, ATP citrate lyase, a cytosolic enzyme involved in the mitochondrial citrate pyruvate shuttle, was also reduced of more than 50 % [71]. In line with these observations, β cell gene expression of several of these molecules was found to be reduced, in comparison with normal β cells [68]. Altogether, these changes in the mitochondria, together with those in glycolysis can explain, at least in part, the reduction of glucose oxidation observed in some studies with type 2 diabetic islets [41,55].

The ATP molecules produced in the mitochondria are then translocated to the cytosol, leading to increased ATP concentrations, that determine closure of the $K_{ATP}^{+}$ channels, decreased $K^{+}$ conductance, and depolarization of the plasma membrane (Fig. 1). This causes the opening of voltage-dependent $Ca^{++}$ channels, followed by the entry of $Ca^{++}$. The influx of the extracellular $Ca^{++}$ into the cytosol mobilizes the insulin granules and favours their exocytosis. Regarding these final steps of insulin release, it has been observed that in type 2 diabetic β cells SUR1 (a key component of the $K_{ATP}^{+}$ channels) was downregulated. In addition, the voltage-dependent calcium channel, L type, alpha 1D subunit (CACNA1D) was also downregulated, whereas potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1), which acts to repolarize cells, was clearly upregulated in β cells from type 2 diabetic individuals [68]. At the protein level, diabetic islets show reduced expression of molecules involved in insulin granule exocytosis, such as those of the SNARE complex and SNARE-modulating proteins (syntaxin-1A, SNAP-25, VAMP-2, Munc 18, Munc 13−1, and synaptophysin) [73] (Fig. 1). Finally, an impairment in exocytotic function at the human single β cell level has been recently demonstrated, with glucose-regulated insulin granules fusion events that are dysrupted in type 2 diabetes [74].

To summarize, several β cell compartments, functions, structures and molecules involved in the mechanisms leading to glucose-stimulated insulin release are impaired in human type 2 diabetes, likely due to the interplay between the genetic architecture of the β cells and the role of environmental factors [12–20,75].

## 5. The pathology of the β cells in type 2 diabetes

The first link between the pathology of the pancreas and diabetes dates back to more than one century ago, when hyaline degeneration of the islets was observed in pancreases that were removed at autopsy from diabetic subjects [76]. Over the past few decades, several studies have been performed on the pancreas from type 2 diabetic patients by the use of autoptic samples, surgical specimens, or the tissue obtained from organ donors [37].

The amount of β cells in non-diabetic and type 2 diabetes individuals can be expressed as β cell mass (when the weight of
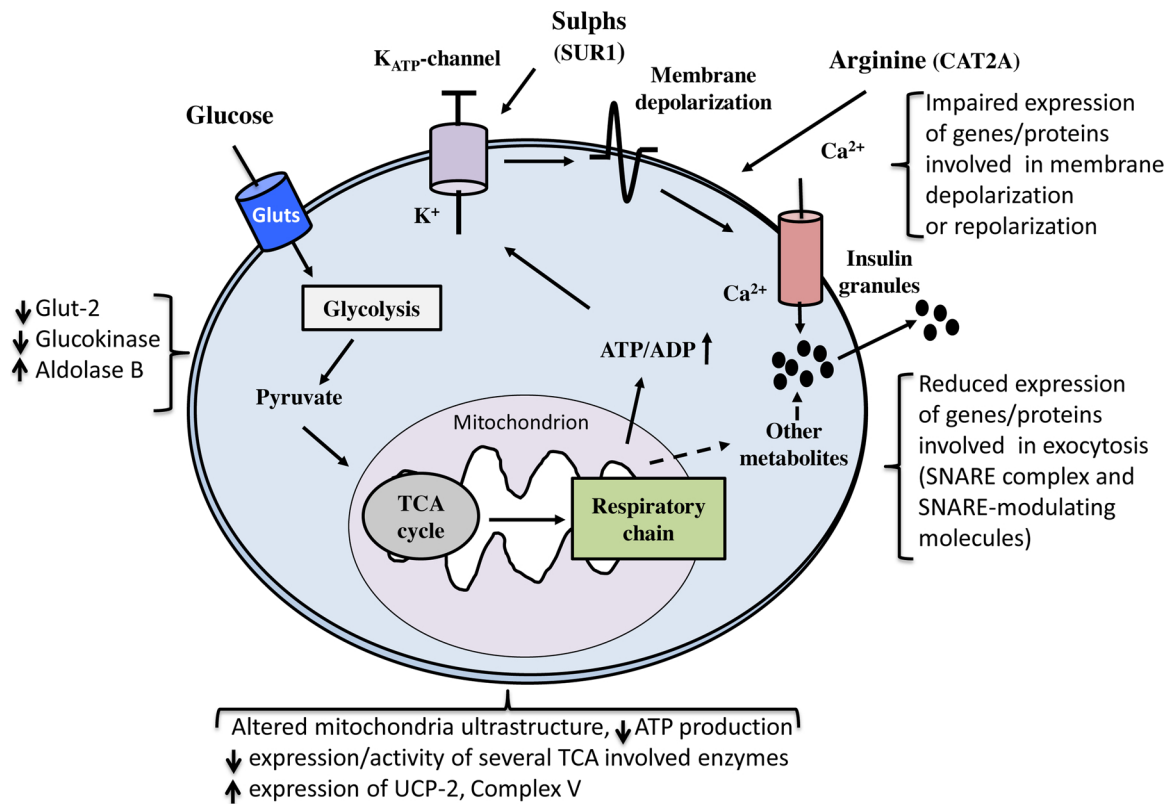
**Fig. 1.** Different insulin secretagogues act at different sites of the β cells. Glucose undergoes extensive metabolism through glycolysis and the TCA cycle to produce ATP and trigger the successive steps leading to the exocytosis of the insulin granules. Sulphonylureas (Sulphs) induce insulin secretion by binding to the $K_{ATP}$ channels, determining their closure and inducing membrane depolarization. Arginine enters the β cells through the CAT 2A cationic aminoacid transporter and causes excess of positive charges and subsequent depolarizaiton of the plasma membrane. Several of these steps are impaired in the type 2 diabetes β cells, and the figure also reports some of the major changes described in the text.

pancreatic samples is available), volume (usually assuming that the islets are spherical) and/or area (insulin-positive proportions in the islets or the pancreatic tissue). Although normally looking islets can be appreciated in the pancreas of type 2 diabetic individuals (Fig. 3), with few exceptions [77,78] β cell amount has been reported to be lower in the type 2 diabetic pancreata [79–89]. Early work showed that the number of islets was 30–50 % lower in type 2 diabetes than control individuals [79,80] and that islet volume was also reduced, which became more apparent when taking into account the presence of amyloid [81]. As discussed in detail elsewhere [82–84] amyloid is derived from islet amyloid polypeptide (IAPP, amylin), a protein that is coexpressed and cosecreted with insulin by β cells. IAPP has the propensity to form membrane permeant toxic oligomers, which seem to contribute to β cell damage [83,84]. In a successive article, a 24 % reduction in β cell area was described in diabetic (n: 15) vs non-diabetic (n: 10) pancreatic samples, that was associated with increased exocrine fibrosis in the diseased pancreata [85]. In Japanese type 2 diabetic individuals, islet β cell volume and total β cell mass were reported to be significantly lower (∼ 30 %) than in non-diabetic controls [86]. Accordingly, a study conducted with pancreatic tissue from organ donors or after surgical removal showed that β cell volume was 25 % lower in type 2 diabetes [87]. A comprehensive study was performed with autoptic samples from non-diabetic subjects, individuals with impaired fasting glycaemia and type 2 diabetic patients (all subdivided into lean or obese) (88). It was found that obese subjects with IFG or diabetes had an approximately 50 % reduction in ß-cell volume, compared with non-diabetic individuals matched for body mass index. In the non-obese group, type 2 diabetes was associated with about 40 % lower β cell volume. In another study, the authors analyzed autoptic samples from 57 type 2 diabetic and 52 non-diabetic European subjects [89]. They showed that on average β cell mass was 35 % lower in the diabetic series.

Intriguingly, however, it was also oberved that there was a large overlap between the β cell mass values of the type 2 diabetes and control samples, suggesting that other factors, in addition to the partial loss of β cells, play a role in the failure of β cells in this form of diabetes. Accordingly, a comparison made with pancreata obtained from lean and overweight/obese diabetic or nondiabetic organ donors [90] found that β cell volume and mass were reduced by approximately 35 % in the obese, but not the lean, type 2 diabetic patients. More recently, a 25 % reduction of β cell areas was confirmed, together with higher amount of islet amyloid, in type 2 diabetic samples [91]. Interestingly, a study performed by combining light and electron microscopy [92] has suggested that the loss of β cells in type 2 diabetes, as assessed by insulin immunostaining, may be overestimated, since degranulated β cells, identified by electron microscopy, may not be appreciated by light microscopy.

To summarize, the quantity of β cells is lower in type 2 diabetes than in non-diabetic pancreata, although there could be some overestimation of the loss. Intriguingly, there is an apparent overlap between the values of β cell mass between diabetic and non-diabetic samples, pointing to the relevant role of β cell functional impairment as a major factor leading to this form of diabetes.

## 6. β cell death and regeneration

The reduced amount of β cells in type 2 diabetes is thought to be due to increased death and reduced regeneration [93–95]. β cell death mainly occurs via apoptosis (Fig. 4), a type of programmed cell death morphologically characterized by cell rounding up, bleb formation and chromatin condensation [96]. As reviewed elsewhere [97–99], apoptosis is a complex process that can be executed by the caspase signaling as well as the Bcl family member/cytochrome C-regulated signaling,
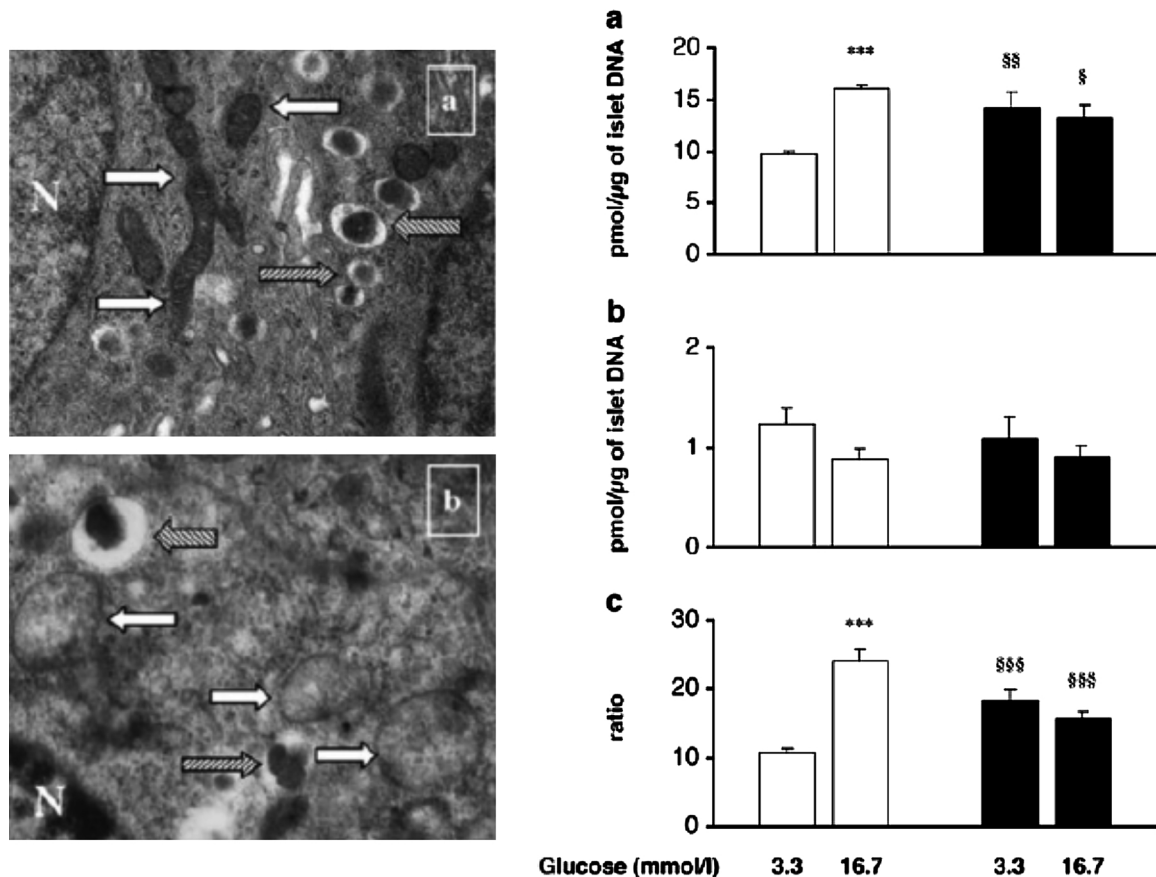
**Fig. 2.** Left panels show the electron microscopy appearance of mitochondria (white arrows) in a normal β cell (upper panel) and a type 2 diabetes β cell (bottom panel); in this latter the mitochondria look less electron dense and their shape is altered as compared to the normal cell; grey arrows indicate insulin granules and N identifies the nucleus. Right panels illustrated ATP production (a), ADP levels (b) and their ratio (c) in non.-diabetic (white bars) and type 2 diabetic (black bars) islet cells in response to low and high glucose, showing lower ATP generation in the diabetic cells. Adapted with permission from Ref. [70].
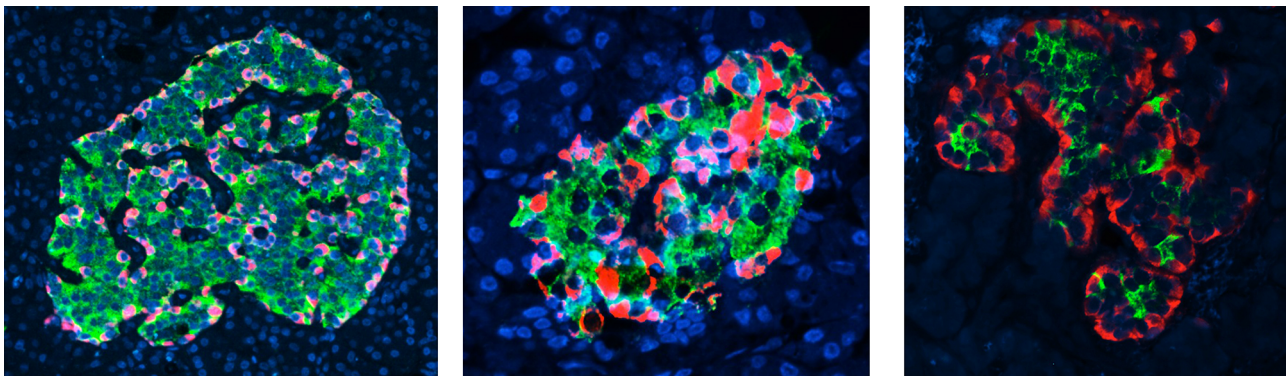


**Fig. 3.** Representative light microscopy images of a normal islet from the pancreas of a non-diabetic donor (left), a normally looking islet (middle) and an islet with reduced number of insulin containing cells (right) from the pancreas of a type 2 diabetic donor. Insulin: green color, glucagon: red color. Courtesy of Mara Suleiman.

acting through caspase 3. By studying autoptic pancreatic samples by the TUNEL technique, apoptosis (expressed as cell/islet and normalized for islet volume) has been shown to be significantly increased in both obese (approximately 0.25 %) and lean (approximately 0.50 %) type 2 diabetic cases in comparison with body mass index-matched non diabetic controls (approximately $0.03 - 0.05\%$) [88]. In another study, a 3-fold increase of β cell apoptosis (0.6 % vs 0.2 % of insulin positive cells) was observed in the pancreas of obese type 2 diabetic patients vs matched controls, although there was no difference in lean diabetic vs non-diabetic samples [90]. In a report in which electron microscopy was used, β cells with signs of advanced apoptosis (such as complete condensation of nuclear chromatin) were approximately 3% in the type

2 vs less than 1% in the non-diabetic samples [100]. Furthermore, the assessment of cytoplasmic histone-associated DNA fragments has demonstrated that islet cell death was significantly higher in islets isolated from type 2 diabetic donors [57]. These changes were shown to be associated with increased numbers of cells positive for activated caspase 3 [101] as well as greater activity of caspase-3 and caspase-8 [57], key molecules in the induction and execution of apoptosis.

However, in addition to apoptosis there are other forms of programmed cell death [89,92]. One of these forms involves macro-autophagy, that is morphologically characterized by massive vacuole accumulation in the cytoplasm [96,99] (Fig. 4). In general, normally functioning autophagy has a beneficial role for cells [95], which
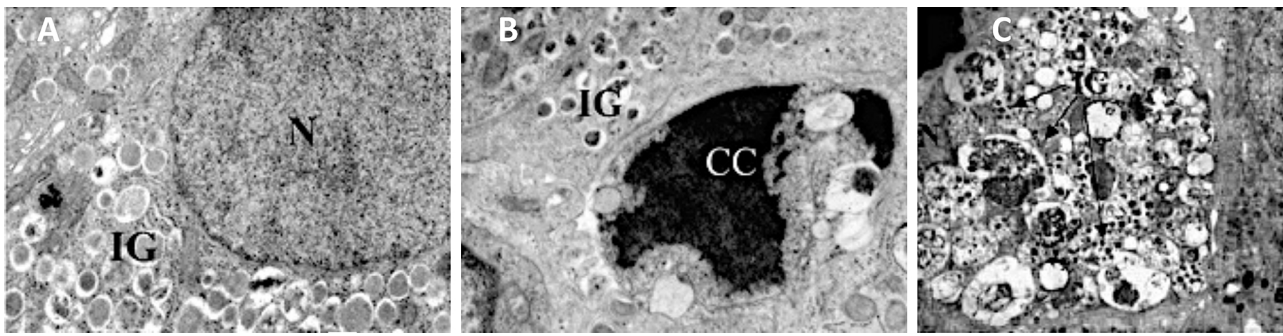
**Fig. 4.** Representative electron microscopy images showing a normal β cell (A), an apoptotic β cell (B; note the marked chromatin condensation, CC, in the nucleus) and a β cell undergoing autophagy-associated death (C; note the cytoplasm engulfed with autophagic vacuoles). IG indicates insulin granules. N: nucleus. Adapted with permission from refs. [60] and [100].

includes the β cells [96–100]. In fact, autophagy accellerates the turnover of aged or damaged proteins and organelles [102]. However, cells that undergo altered autophagy may die in a non-apoptotic manner [102–107]. The presence and role of autophagy in β cells has been investigated in several models, comprising human pancreatic samples. When > 4000 endocrine cells were studied in type 2 diabetic and non-diabetic pancreata, dead β cells with massive vacuole overload and no major chromatin condensation (morphological evidences of autophagy-associated cell death) accounted for 1.18 % and 0.4 % respectively in the diabetic and non-diabetic condition [60]. Interestingly, this was associated with reduced gene expression of LAMP2, Cathepsin B and Cathepsin D, all involved in the autophagic flux [60]. Therefore, both apoptosis and autophagy-associated β cell death contribute to the reduction of β cell amount in type 2 diabetes.

Defects of β cell regeneration may also play a role in the reduction of β cell mass in human type 2 diabetes, but this issue is still unclear. In principle, β cell regeneration may essentially occur by replication (proliferation) of existing cells, neogenesis from precursors or trans-differentiation of other, existing mature cells, as essentially shown in non-human experimental models [108–110]. In human adults, these processes are likely to occur at a very low rate [111,112]. Nevertheless, β cell mass can increase in case of obesity and during pregnancy, suggesting possible regeneration events [113,114]. This issue has been explored at the histological level in a few studies with type 2 diabetic pancreata. By using Ki67 staining as a marker of proliferation and the presence of insulin positivity in duct cells as an index of neogenesis, it has been reported a similar frequency of β cell replication and neogenesis rate between obese or lean type 2 diabetic patients and the body mass index-matched non-diabetic groups [88]. Both stainings were identified in a very low proportions of cells: 0.025 % per islet, normalized per islet volume (Ki67 staining) and 0.040 % (duct cells positive for insulin). Another study reported slightly different findings [90]. β cell replication was assessed by proliferating cell nuclear antigen (PCNA) staining and resulted lower in overweight/obese type 2 diabetic than in weight-matched non-diabetic individuals. Then, the authors calculated a neogenic index, base on the amount of insulin positive cells or small clusters in the duct walls and/or the acinar tissue; this index was similarly higher in non-diabetic obese individuals and in obese or lean type 2 diabetic subjects, in comparison with the non-diabetic lean subjects [84]. Whereas lack of differences in signs of β cell replication has been confirmed in other investigations [115], a study has reported that the proportion of insulin-positive cells in duct walls was significantly more elevated in subjects with impaired glucose tolerance and significantly lower in those with type 2 diabetes, compared to nondiabetic cases, possibly indicating an attempt of compensation in the prediabetic state, that is lost after the onset of diabetes [116]. These results, obtained with the use of surrogate markers and indexes of regeneration, show that β cell replication does not seem to differ between type 2 diabetic and non-diabetic β cells, whereas neogenesis may be reduced in type 2 diabetes.

## 7. Trans- and de-differentiation

As mentioned above, trans-differentiation is a process through which β cells can derive from other, different mature cells, thus contributing to the modulation of β cell mass [110,117–119]. Sophisticated experimental studies have demonstrated that α cells, producing glucagon, and δ cells, producing somatostatin, evaluated *ex vivo* after preparation from human islets, can transdifferentiate into β cells [117,119]. Although such experiments are not directly applicable in human subjects, histological examination of the human pancreas has revealed the presence of cells containing both glucagon and insulin, suggesting pancreatic endocrine cell plasticity and possible cell type interconversion [120,121]. Some authors have shown that at weeks 13–25 of gestation a proportion of pancreatic endocrine cells (up to 8–9 %) co-express insulin and glucagon [122]. However, these double insulin and glucagon positive islet cells are rare in adults [122–124]. In the pancreas of individuals with insulin resistance or altered glycemic values an increased amount of cells expressing both hormones has been described. A study with 16 non-diabetic subjects was performed by the use of a 2 h hyperglycemic clamp and β cell secretory function assessed. Pancreatic samples obtained from the same patients during pancreatoduodenectomy were evaluated to examine glucagon and insulin double positive cells [123]. It was found that the proportion of bihormonal cells (containing both insulin and glucagon) was significantly higher in insulin resistant subjects (16 %) than in insulin sensitive individuals (9 %). When the pancreas of subjects with normal glucose values, impaired glucose tolerance (IGT), newly diagnosed or longstanding type 2 diabetes were examined, the freuence of insulin and glucagon double positive cells was respectively 0.45 %, 0.62 %, 0.82 % and 0.33 %, indicating increased proportions of such cells in IGT and recently onset diabetes, but not when the disease was long-lasting [116]. However, in another report [91], confocal microscopy analysis showed that bi-hormonal cells were more common in a type 2 diabetic group than in control subjects (4.0 % vs 0.5 %) with the finding confirmed by flow cytometry of dispersed human islets. Finally, electron microscopy assessment of pancreatic islets in non-diabetic and type 2 diabetic islets corroborated these previous observations, showing that the latter had more cells containing both insulin and glucagon granules (2.4 % vs 1.2 %) [124]. Of interest, it has been also found that in the pancreas from some type 2 diabetic donors there are cells with both zymogen-like and insulin-like granules, scattered in the exocrine compartment [118]. These cells ranged from 0.82 to 1.74 per $mm^2$, corresponding to 0.26 ± 0.045 % of the counted exocrine cells. Similarly, cells containing both carboxypeptidase A and insulin were more frequently observed by immunohistochemistry in the diabetic pancreata [125].

Based on work performed with cell lines and rodent models

[126–130], de-differentiation of β cells has been recently proposed as mechanism possibly explaining both insulin secretion dysfunction and reduced amount of insulin containing cells in type 2 diabetes. The concept of de-differentiation implies that under given stressful conditions, including gluco- and/or lipotoxic insults [130–132], β cells may loose their molecular and functional identity, becoming functionally incompetent and, over time, also more prone to death. The transcription factor FoxO1, that is key to maintain β cell identity, has been proposed as a fundamental player in these processes [126,128]. In healthy β cells FoxO1 resides in the cytoplasm, but during initial stressful events it translocates into the nucleus to enforce β cell identity. However, if the stress prolongs, FoxO1 expression declines, Neurogenin 3, Octamer-binding transcription factor 4 (Oct4), Homeobox protein Nanog, and L-Myc are reactivated, all this leading to a drop in insulin production and secretion. Then, former mature β-cells may revert to an uncommitted endocrine progenitor stage, and some may undergo conversion into other hormone-producing cells. A few data on this issue have been generated with also the human pancreas [133,134]. In one study [133], the analysis of 15 control and 15 diabetic donors was performed. Markers of endocrine lineage, β cell-specific transcription factors, and aldehyde dehydrogenase 1A3a (an endocrine progenitor cell marker) staining were used. Results showed that de-differentiated cells were almost 4-fold more common in the type 2 diabetic samples (31.9 vs 8.7 %), The number of aldehyde dehydrogenase 1A3-positive/hormone-negative cells was 3-fold higher in diabetics compared with controls. In addition, in samples of type 2 diabetic patients β cell-specific transcription factors were ectopically found in glucagon- and somatostatin-containing cells. Interestingly, it was observed that at higher degree of de-differentiation corresponded lower glucose-stimulated insulin release [133]. In another study, however, much fewer cells with clear signs of de-differentiation were reported [134].

In conclusion, plasticity of β cells in the human pancreas has been inferred by histological evidence, with hints of trans- and de-differentiation; these features differ quantitatively between type 2 diabetic and non-diabetic subjects, suggesting a role in type 2 diabetes pathophysiology.

## 8. The role of inflammation

Evidence indicates that pancreatic islet cells in human obesity and type 2 diabetes may show signs of inflammation, including immune cell infiltration, as well as increased expression of cytokines and chemokines [135–140]. In type 2 diabetes, an early study examined the presence of macrophages in 7 non-diabetic subjects and 9 patients with the disease [141]. It was shown that the percentage of macrophage (CD68+, > 3 per islet) infiltrated islets was 16 % in the diabetic group, corresponding to a 4-fold increase versus non-diabetic samples. In addition, the number of macrophages per islet was higher in the diseased islets in comparison with control tissue (1.7 vs 0.7), with also more marked intra-islet infiltration [141]. Afterwards, the comparison of 15 diabetic and 16 control autoptic pancreatic samples confirmed a higher proportion of islets with macrophage infiltration and an increased number of CD8+ cells per islets in the diabetic glands [142]. In another study, the authors found greater macrophage infiltration in amyloid expressing islets [143]. Finally, an investigation mainly focused on type 1 diabetes has reported that in type 2 diabetes also the acinar tissue shows increased macrophage infiltration, indicating that inflammation features might affect not only the endocrine islet cells but also the exocrine compartment [144]. As for other cells of the immune system, islet lymphocytes have been reported to be or not to be increased in pancreatic samples from 2 diabetic subjects [141,144], whereas no difference in the amount of granulocytes has been observed between diabetic and non-diabetic pancreatic islets [141].

The presence of islet inflammation in type 2 diabetes has also been investigated at the molecular level. In a first study, the use of microarray analysis to evaluate the trascriptome of isolated islets in a small group of type 2 diabetic donors did not show any difference in comparison with non-diabetic islets as for the expression of the major cytokines and chemokines [66]. However, a report published afterwards showed that the transcriptomes of islets prepared from six type 2 diabetic and seven non-diabetic organ donors were characterized by differential expression of numerous genes, which included upregulation of a few cytokines (IL-19, IL-25, CSF1, LTA and IFNB1) and chemokines (CCL5, CCL7, CCL13, CCL16, CCL21, CCL25 and CX3CL1) [145]. Microarray data generated successively revealed that two proinflammatory chemokines, CCL22 and CXCL5, were downregulated in the diabetic islet samples [67]. In addition, another study by the same group found upregulation of the proinflammatory cytokine IL-6 and the immunomodulatory cytokines IL-11 and IL-33 in the islets prepared from type 2 diabetic donors [146]. These findings have been implemented in a recent, comprehensive study conducted with a large number of islets from non-diabetic (n: 84) and type 2 diabetic (n: 19) organ donors by Affymetric microarrays [56]. The study showed upregulation of a few cytokines/cytokine receptors (IL1β, IL7R, IL17R) and several chemokines/chemokine ligands (such as CCL3, CCL8, CXCL2, CXCL11, CXCL12). Of interest, a report was published on the transcriptomes of β cells yielded by laser capture microdissection from 10 non-diabetic and 10 type 2 diabetic pancreata [68]. The results revealed that in type 2 diabetes samples IL1β and IL8 expression signals trended higher, IL11 was upregulated and IL1α was downregulated, and CCL2, CCL11 and CCL13 were the most upregulated chemokines.

Information on the expression of inflammatory mediators in human islet cells at the protein level is limited. IL1β abundance was studied in pancreatic samples from five patients with poorly controlled type 2 diabetes [147]. Double immunostaining for this cytokine and insulin evidentiated the presence of IL1β in clusters of insulin-containing cells in approximately 20 % of the studied islets. In another study, chemokine (C-C motif) ligand 2 (CCL2) staining colocalised with insulin in both type 2 and non-diabetic samples, with minimal or no CCL2 in glucagon-positive cells [148]. However, quantitative assessments were not performed.

Therefore, studies have shown that type 2 diabetic islets show higher infiltration by immune cells, in particular CD68+ macrophages, than control islets [138,140,149]. This accumulation is thought to arise by local proliferation of resident macrophages [138,140] and could legitimate the presence of immune mediators in the islet cells. However, some cytokines and chemokines could be also directly produced be the β cells, particularly in a pro-inflammatory environment [68,147,148]. All this may be responsable, at least in part, of β cell dysfunction and death. However, it should be taken into account that macrophages also have beneficial effects on islet cells, contributing, for instance, to pancreas development and, under certain circumstances, to insulin secretion [150,151]. Similarly, the overall effects of cytokines on human β cell function and survival depend on other factors, including, in particular, their concentrations and combinations. For example, low levels of IL-1β improve insulin secretion and promote β cell proliferation and [152]; furthermore, when human β cell preparations were cultured for 48 or 72 h with or without IL1β, TNFα or IFNγ, none of these single cytokines was toxic to the β cells [153].

## 9. Conclusions

In this review we have focused on several features of β cell dysfunction and pathology in human type 2 diabetes. We have shown how the key *in vivo* insulin secretion defects documented in this disease can be recapitulated *ex vivo* by the direct study of isolated islets, which also allows to assess crucial biochemical and molecular traits associated with the functional impairment (Table 2). Pathology studies have revealed fundamental β cell characteristics in type 2 diabetes, such as alterations of β cell quantity and turn over as well as the presence of inflammatory traits, and suggesting unexpected β cell plasticity, that might comprise trans- and de-differention as possible attempts to adapt

**Table 2**
Major β cell dysfunction and pathology features in human type 2 diabetes.

| Feature | Changes in type 2 diabetes |
| --- | --- |
| Glucose-stimulated insulin secretion | Reduced; loss of first phase release |
| Other nutrient-stimulated insulin secretion | Less severely affected |
| β cell mass | Reduced of approximately 30 % on average, mainly due to increased death by apoptosis and altered autophagy-associated demise; possibly overestimated in some cases |
| β cell regeneration | Low; not clearly reduced in comparison to non-diabetic samples, but anyhow insufficient to reconstitute the mass of β cells; transdifferentiation form other islet cells suggested |
| β cell de-differentiation | Increased (relevance debated) |
| Inflammation | Increased macrophage infiltration and cytokine/chemokine expression (debated; better phenotypic characterization of infiltrating macrophages needed) |

to metabolic stress (Table 2). However, it has to be kept in mind that type 2 diabetes is clinically a quite heterogenous disease itself [11,154], and several variables may impact the quality of the pancreatic tissue and isolated islet cells (tissue source: autoptic, surgical, bioptic, from organ donors; islet preparation: isolation stress, culture conditions and duration) [37,45,58]. Therefore, correct reporting of islet data may be challenging [7], leading to potential inconsistencies in the experimental results. Despite these at the moment only partially unavoidable limitations, the information presented in this review provides pathophysiological evidences and clues to conceive new strategies for better prevention and treatment of type 2 diabetes by targeting the β cells.

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