β-cell apoptosis in type 2 diabetes: quantitative and functional consequences

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Abstract

Type 2 diabetes, the most common form of diabetes in humans, is characterized by impaired insulin secretion paralleled by a progressive decline in β-cell function and chronic insulin resistance. Several authors have showed that in type 2 diabetes there is a reduction of islet and/or insulin-containing cell mass or volume. Regulation of the β-cell mass appears to involve a balance of β-cell replication and apoptosis but, at the molecular level, pancreatic β-cell loss by apoptosis appears to play an important role in the development of insulin deficiency and the onset and/or progression of the disease. The mechanisms favoring apoptosis in type 2 diabetic pancreatic islets and new potential therapeutic approaches to prevent β-cell death and maintain β-cell mass are discussed.

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Résumé

Apoptose des cellules β-pancréatiques dans le diabète de type 2 : conséquences quantitatives et fonctionnelles

Le diabète de type 2, la forme de diabète la plus fréquente chez l’homme, est caractérisé par une altération de la sécrétion de l’insuline s’accompagnant d’un déclin progressif de la fonction β-cellulaire et par une insulinorésistance chronique. De nombreux auteurs ont montré que dans le diabète de type 2, il existait une réduction des îlots et/ou de la masse ou du volume des cellules contenant l’insuline. Il apparaît que la régulation de la masse β-cellulaire implique un équilibre entre la réplication et l’apoptose des cellules β, mais qu’au niveau moléculaire, la perte des cellules β-pancréatiques due à l’apoptose jouerait un rôle important dans le développement de la déficience insulinaire et l’apparition et/ou la progression de la maladie. Les mécanismes favorisant l’apoptose des îlots pancréatiques dans le diabète de type 2 et les nouvelles approches thérapeutiques potentielles pour prévenir la mort des cellules β et le maintien de la masse β-cellulaire sont discutés dans cette revue.

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Keywords: Pancreatic β-cells; Apoptosis; Type 2 diabetes

Mots clés : Cellules β-pancréatiques ; Apoptose ; Diabète de type 2

1. Introduction

Type 2 diabetes is a disease characterized by progressive worsening of glycaemic control. It usually starts with mild alterations in postprandial glucose homeostasis, followed by increase in fasting plasma glucose. Upon diagnosis and initiation of antidiabetic therapy, glycaemic control either continues to worsen or more intensive and complex treatment has to be implemented [1]. The nature of this progression must reflect changes in the underlying pathogenetic mechanisms. It is well recognized that Type 2 diabetes is the result of two major defects: insulin resistance and defective β-cell function [2]. These two defects, however, contribute to a different extent to the development of the disease. Insulin resistance is likely to be almost maximal already in the early stages of the disease. During this phase of the disease glucose tolerance is maintained at the expense of increased insulin secretion, so that insulin-resistant individuals are characteri-
zed by compensatory hyperinsulinemia [3, 4]. As soon as compensatory insulin hyper-secretion declines impaired glucose tolerance and overt diabetes develop. Hyperglycaemia and poor metabolic control may then worsen insulin sensitivity, but it is the progressive decline of β-cell function that sets the pace for increased treatment demand and continuous increase in plasma glucose levels.

The results of the United Kingdom Prospective Diabetes Study (UKPDS) have translated into the clinical paradigm of the natural history of type 2 diabetes [5]. The study has shown some crucial features of the development and progression of the disease. Newly diagnosed Type 2 diabetic patients were enrolled in the trial and in all of them two simple, though validated, measures of insulin action (HOMA-IR) and β-cell function (HOMA-B) were obtained to show that the latter was already reduced by 50% at the time of diagnosis. In the subsequent follow-up no much change occurred in HOMA-IR independently of treatment patients were allocated to [6]. Some change was, on the contrary, observed for HOMA-B in response to different treatments with an initial increase in sulfonylurea treated patients. However, following this initial improvement, HOMA-B begun declining again at a rate similar to the one of patients on diet or metformin. The rate of the decline was so linear that the UKPDS investigators speculated it was possible to extrapolate back to the time the loss of β-cell function actually started.

A slightly revised description of the natural history of β-cell function has been proposed by the Belfast Diabetes Study [7]. According to this model, subjects who will develop diabetes already have limited β-cell function to start with. Moreover, loss of β-cell function recognizes two phases: phase A) preceding overt diabetes, characterized by a slow but constant decline (2% per year), and phase B) mainly occurring after development of overt hyperglycaemia with a significant acceleration (18% per year) in the worsening of insulin secretion. While phase A may reflect intrinsic defects of the β-cell, acceleration of phase B is the likely consequence of gluco- and lipotoxicity, suggesting once diabetes becomes manifest a vicious cycle may develop.

2. Mechanisms of loss of β-cell mass and function in Type 2 diabetes

The decline of insulin secretory function is paralleled by progressive reduction of β-cell mass, while increased demand would be expected to increase the number and the volume of insulin-producing cell. A reduction of β-cell mass has been reported in patients with type 2 diabetes though the exact figure is far from being clear. Guiot et al. found reduced β-cell mass in lean or obese patients requiring insulin therapy, but not in obese type 2 diabetic patients on diet therapy [8]. Other studies [9,10] have shown normal or even increased β-cell mass. Analysis of electron microscopy of islets obtained from pancreas of multi-organ donors who turned out to have type 2 diabetic, yield, in our hands [11], to a modest reduction of β-cells as compared to islets from non-diabetic donors (61% vs. 69%) together with a slight increase in α-cells (25% vs. 21%). This reduction in β-cell number was associated with increased apoptosis and increased expression of caspase 3 and 8, both apoptotic mediators [12].

Relative β-cell volume was found to be increased in obese versus lean non-diabetic subjects via increased neogenesis [13]. Compared with people whit no diabetes, obese individuals with impaired fasting glucose and type 2 diabetes had a 50% and 63% reduction in β-cell mass, respectively, whereas lean diabetic had a 41% deficit. In all cases, low rates of cell replication were observed, with no difference between diabetic and non-diabetic subjects. In contrast, the frequency of β-cell apoptosis was 10-fold greater in lean and 3-fold greater in obese individuals [14]. These results suggest that pancreatic plasticity is an important mechanism to meet increased homeostatic insulin demand of insulin-resistant obese individuals. In addition, these findings indicate that in type 2 diabetes, a relative reduction of β-cell mass occurs as the result of excessive apoptosis. As already recalled, however, this reduction in β-cell mass and function is apparent already in the presence of impaired glucose tolerance, suggesting that an intrinsic defect exists in pancreatic islets. Likely enough this reflects genetic predisposition, while the rate of loss of β-cell may be affected by concomitant environmental conditions.

It is conceivable that intrinsic defects of the β-cell leads to inability to meet increased demand due to concomitant insulin resistance. Physiological fluctuations in the metabolic milieu should indeed result in increased insulin secretory capacity through activation of complex adaptive processes such as modulation of β-cell mass, resetting of β-cell threshold of response to stimuli, and increased insulin biosynthesis and release [15,16]. Regulation of β-cell mass is a dynamic process and represents the balance between expansion and involution (shrinkage) of β-cells (Fig. 1) [9]. The number of β-cells can increase (hyperplasia) by active replication and/or neogenesis. This increase is balanced by reduction in cell number via cell death that may results from necrosis and/or apoptosis. Apoptosis that plays a critical role in the reduction of β-cell mass occurring in patients with type 2 diabetes. Butler et al [18] reported that β-cell mass is reduced by 50% in subjects with impaired fasting glucose and by 63% in patients with type 2 diabetes. This reduction was entirely accounted for by increased apoptosis, since regeneration was similar in control and type 2 diabetic islets.

Apoptosis, or programmed cell death, is a coordinated series of events for the programmed execution of cell death, and plays an important role in the maintenance of tissue homeostasis through activation of an intrinsic suicide program. This is a physiologic relevant process as it aims at removing infected or mutated cells from the pancreatic islet.
In summary, the progressive loss of functional β-cell mass accounts for progression toward overt diabetes and subsequent progressive worsening of glycaemic control. This phenomenon is the consequence of imbalance between β-cell replication and/or neogenesis and apoptosis with the latter constantly found to be much activated.

3. β-cell apoptosis via extrinsic and intrinsic complex signaling pathways

β-cell apoptosis can occur in response to several insults acting through at least three pathways leading to destruction of cell chromosomes. These pathways include: i. cytokine-induced cell death, mediated by cell surface death receptors [19,20], ii. mitochondrial disruption, for example, secondary to oxygen free radicals [21,22], and iii. endoplasmic reticulum (ER) stress pathway [23,24]. The human pancreatic β-cell is vulnerable to all three forms of apoptosis and in type 2 diabetes they can be triggered by metabolic alterations, i.e. glucotoxicity and lipotoxicity.

4. Glucotoxicity

Progressive increase of plasma glucose levels characterizes the natural history of type 2 diabetes. Several studies have shown that elevated blood glucose concentration impairs β-cell function and insulin sensitivity, a phenomenon known as glucotoxicity [25]. Incubation of human pancreatic islets in the presence of high glucose concentrations has repeatedly documented an increase of apoptotic rate. Several mechanisms have been claimed to increase β-cell programmed death. A common and powerful mechanism is the activation of oxidative stress due to increased mitochondrial generation of reactive oxygen species (ROS) that follows excessive glucose metabolism. The β-cell is quite sensitive to oxidative stress due to very low expression and activity of anti-oxidant enzymes. In our laboratory, Del Guerra et al have analyzed the degree of oxidative stress in pancreatic islets obtained from the pancreata of cadaveric donors with or without type 2 diabetes [111]. The levels of markers of oxidative stress such as nitrotyrosine and 8-hydroxy-2′-deoxyguanosine were much greater in diabetic islets than in the control ones. Moreover, an inverse correlation was found between intracellular concentration of the two markers and glucose-stimulated insulin release. Addition of glutathione, an anti-oxidant compound, to the incubation medium lowered nitrotyrosine levels, recovered much of the secretory function, and increased insulin mRNA expression. More recently, we have shown that even intermittent high glucose levels hamper glucose-stimulated insulin secretion, activate apoptosis, cause alteration of mitochondrion morphology and density volume, and are associated with increased intracellular nitrotyrosine content [26]. These findings are of interest as they suggest that even glucose fluctuations, as they may occur in response to meal ingestion in the prediabetic conditions, may accelerate the loss of functional β-cell mass.

Under condition of increased glucose availability, not only oxidative stress is enhanced, but other pathways are activated, including hexosamine biosynthesis. Glutamine: fructose-6-phosphate-aminotransferase (GFAT) is the rate-limiting enzyme of this pathway, catalyzing the conversion of fructose-6-phosphate to glucosamine-6-phosphate (GlcN-6-P) with glutamine as amino donor. GlcN-6-P is rapidly converted and activated to uridine-5-diphosphate-N-acetyl-glucosamine (UDP-GlcNAc), a precursor for the synthesis of the carbohydrate moiety of glycoproteins, glycolipids, and proteoglycans. In vitro, increased activity of this pathway in β-cell is associated with increased apoptosis [27]. Moreover, incubation of pancreatic islet with high levels of glucosamine, the product of the hexosamine pathway, results in impaired activation of the insulin receptor/IRS/PI3-kinase/Akt survival pathway. These observations are well in keeping with the important role the insulin signaling pathway appears to exert in the function and survival of β-cell [28].

The role of cytokines in the pathophysiology of Type 1 diabetes is well recognized [29,30] but they are mediators of glucose toxicity in type 2 diabetes [31,32]. Glucose- and cytokine-activated apoptosis involve intrinsic and extrinsic death pathways mediated by increased expression of cell surface Fas(CD95) as well as cytokine-mediated activation of caspases-8. Moreover, up-regulation of the Fas receptor by elevated glucose levels can contribute to β-cell destruction through constitutively expressed Fas, independent of an autoimmune reaction, thus providing a link between loss of β-cell mass in type 1 and type 2 diabetes [33,34].

Chronic hyperglycaemia results in chronic β-cell stimulation and insulin biosynthesis can activate endoplasmic reticulum (ER) stress [35]. ER is responsible for the synthesis, modification and delivery of proteins to their target sites. These processes may be impaired under various physiological and pathological conditions, leading to ER stress [23,37]. When upregulation of the ER folding capacity through modulation of chaperones andfoldases, downregulation of the biosynthetic load, and increased clearance of unfolded proteins through promotion of ER associated degradation [23,37] cannot meet stress conditions, then apoptosis is ini-
tiated. Marchetti et al. [38] have recently reported that β-cell of type 2 diabetic subjects have only modest signs of ER when pancreatic islets are incubated in the presence of normal concentration of glucose. However, exposure to higher glucose levels caused much greater increase of ER stress markers as compared to control islets [38].

5. Lipotoxicity

Obesity is the main risk factor for the development of diabetes. It is often part of the metabolic syndrome and is accompanied by dyslipidemia and increased circulating levels of leptin and cytokine. Expansion of the adipose tissue is indeed associated with an inflammatory response characterized by increased release of leptin, TNF-α, IL-6 and IL-1 receptor antagonist. These factors have well recognized effects on insulin sensitivity [39] but may also have an effect on β-cell function and survival. Although in rodents leptin has been shown to stimulate β-cell proliferation and protect from FFA-induced apoptosis, exposure of human pancreatic islets is associated with increased apoptosis [40].

Whereas some free fatty acids (FFA) and lipoproteins may exert a pro-apoptotic effect, others appear to be protective. Thus, long-term exposure to saturated fatty acids such as palmitate is associated with toxic effect (Fig. 2), whereas mono-unsaturated fatty acids, such as oleate, protect against palmitate- and glucose-induced β-cell apoptosis [41,42]. FFA activates β-cell apoptosis in a caspase-dependent manner and by down-regulation of Bcl-2 mRNA expression [42]. In addition, FFA-induced apoptosis is linked to down-regulation of Akt phosphorylation. In contrast, activation of Akt inhibits the activation of apoptosis machinery exerting a restraining effect on Bad, cytochrome c release and caspase-9 [43]. Interestingly, functional defect in Akt predisposes to β-cell apoptosis and development of diabetes in the presence of defective insulin signaling [44]. The Zucker diabetic fatty fa/fa rat, islets contain 100-fold higher number of β-cell than in lean rats. However, culture of islets from pre-diabetic Zucker rat in the presence of 1 mM FFA is associated with significant increase in apoptosis. This effect was associated with an increase in the sphingomyelinase product ceramide. It is this compound that may directly trigger apoptosis since when its synthesis is blocked FFA-induced apoptosis is prevented.

FFAs also induce NO synthase expression and cause a fourfold increase in NO production. Again, the use of inhibitors of inducible NO synthase (iNOS) minimizes loss of insulin secretion [45]. Lipotoxicity is strictly linked to glucotoxicity and, according to Poitout and Robertson [46], the former is unlikely to occur in the absence of the latter. In effect, both can activate excessive generation of ROS, which funnel their negative effects through generation of oxidative stress.

6. Amyloid

Islet-amyloid polypeptide (IAPP), or amylin, is a normal secretory product of the pancreatic β-cell and is co-localized with insulin in secretory granules [47]. Islet amyloid is formed primarily by deposition of IAPP in the form of fibrils; the exact mechanism for amyloidogenesis, however, remains unknown. A potential role for IAPP in the development of IR, β-cell dysfunction, and type 2 diabetes has been proposed [48, 49] though these studies have yielded conflicting and inconclusive results [50-52]. In contrast to studies that found no association between amyloid deposits and duration of type 2 diabetes [53], others have shown an association between deposits and apoptosis, replacement of β-cell mass, and decline in β-cell function [54-56]. Some investigators have concluded that up to 90% of patients with type 2 diabetes have amyloid deposits in their islets [57] and the degree of amyloidosis correlates with duration and severity of the disease [58]. Human amyloid is toxic to β-cells [59] and contributes to loss of β-cell mass [60]. In an animal model, islet amyloidosis shows diffuse distribution throughout the pancreas, with a progressive diminution in endocrine mass as amyloid mass increases [61]. In other words, as amyloid deposits increased, β-cell mass shrinks, resulting in impaired β-cell function and glucose intolerance [61].

7. Sulfonylureas and secretagogues

In the UKPDS, glibenclamide and chlorpropamide treatment was associated with improvement in glycaemic control and lower incidence of microangiopathic complications [62]. Nonetheless, as already recalled, improvement of glycaemic control was associated with an initial increase of the HOMA-B, an index of β-cell function, followed by a progressive linear decline [63]. Loss of β-cell mass and function has raised concern regarding the use of sulfonylureas for the treatment of type 2 diabetes. Animal and cell studies indicate that these agents may induce β-cell apoptosis [65sw]. Studies [65] performed in isolated human islets sug-
gest glibenclamide, but not repaglinide, could activate β-cell apoptosis. Nateglinide at low concentrations did not induce β-cell apoptosis, although a 1.5-fold increase in the number of apoptotic β-cells was observed at higher concentrations. After 4-day islet exposure to secretagogues, β-cell apoptosis was apparent for all agents. Incubation of pancreatic islets and β-cell cells from ob/ob mice and Wistar rats with glucose and sulfonfonylureas was also shown to induce apoptotic β-cell death [66].

In our lab, we have assessed glucose-stimulated insulin release, insulin content, islet cell apoptosis, and mRNA expression of insulin and GLUT-1 in isolated human islets cultured in the presence of therapeutic concentrations of gliclazide (10 μM), glibenclamide (10 μM), or chlorpropamide (600 μM) [67]. Insulin content decreased significantly upon exposure to each of these sulfonfonylureas. Insulin responsiveness to glucose was preserved in islets incubated with gliclazide, but not in islets incubated with glibenclamide or chlorpropamide. These observations indicate that difference may exist among sulfonfonylureas with respect to function and survival of cultured human islets. How this translates in vivo is difficult to establish. In the UKPDS, loss of β-cell function was not unique to sulfonfonylureas, as it occurred at the same rate in patients treated with metformin or conventional (mainly diet) treatments, suggesting other factors than treatment contribute to the process.

8. Apoptosis, β-cell loss and functional consequences

The normal pancreas contains approximately 1 million islets of Langerhans, and each islet includes β-cells (60-80%), α-cells (20-30%), somatostatin secreting (δ-) cells (5-15%), and pancreatic polypeptide (PP-) cells. As mentioned, β-cell mass is regulated by apoptosis, hypo- and hyperplasia, replication and neogenesis [68,69]. In other words, regulation of β-cell mass is a dynamic process where the actual mass represents the net balance between replication, growth, and neogenesis on one side and necrosis/apoptosis on the other one. The phenomenon is also known as β-cell plasticity and allows adaptation to changes in demand of β-cell function [70]. Such process is disrupted in type 2 diabetes where functional defects and decreased β-cell mass coexist.

Both impaired proliferation and increased apoptosis may contribute to loss of β-cell mass. Increased apoptosis has been observed in Zucker diabetic fatty rats (ZDF), an animal model of type 2 diabetes [71]. In these animals, expansion of β-cell mass in response to insulin resistance was shown to be inadequate. However, no defects in proliferation or neogenesis could be identified, suggesting excessive rate of cell death by apoptosis could play a major role [72,73]. It is difficult to distinguish between the two mechanisms, cell formation and cell death, in human tissue sections because dead cells are rapidly removed from the islet by macrophages and neighboring cells, making it hard to quantify cell death. Nonetheless, apoptosis is currently believed to represent the main cause for loss of β-cell mass in type 2 diabetes. This view is supported by autopic data where pancreatic tissues from type 2 diabetic patients have been compared to those from non-diabetic subjects [74]. Moreover, elevated activities of apoptotic mediators caspase-3 and -8 have been found in β-cells from islets of type 2 diabetic patients [75].

The impact of a reduction of β-cell mass in the alterations of insulin secretion that characterize type 2 diabetes has not yet fully elucidated. Still, clinical observation and experimental data support a close interrelationship between the two parameters. Thus, a large proportion of living related pancreatic donors who underwent a 50% pancreatectomy developed diabetes [76]. Pharmacological or surgical reduction of β-cell mass in rodents [77], pigs [78], dogs [79] and primates [80] results all in impaired insulin secretion. More recently, Matveyenko et al [81] have carefully analyzed the effect of 50% pancreatectomy in normal dogs to show that partial pancreatectomy resulted in IFG and IGT. Partial pancreas resection was associated with reduction of both basal and glucose-stimulated insulin secretion. Altogether these data support a mechanistic role of reduced β-cell mass in the development of alterations in glucose homeostasis and progression toward type 2 diabetes.

In conclusion, it looks like the major defect leading to decreased β-cell mass in type 2 diabetes is inappropriate apoptosis, while new islet formation and β-cell replication are normal so that therapeutic approaches designed to arrest apoptosis could have quite an impact on prevention and treatment of the disease.

9. Targeting β-cell apoptosis

Maintenance of β-cell mass in people at risk of diabetes or in those who already have developed clinical hyperglycaemia requires understanding of the mechanisms regulating the dynamic interaction between proliferation/regeneration and cell death. Many factors have been so far identified [82], but among them great interest has been generated by the therapeutic role of glucagon-like peptide-1 (GLP-1). GLP-1 is an incretin secreted by the L-cells of the gut. It was recognized many years ago that insulin response following oral ingestion of glucose was much greater than the one elicited by a similar i.v. glucose load [83]. While this effect may account for up to 60% of postprandial insulin secretion of normal individuals, in patients with Type 2 diabetes it is quite reduced [84]. The incretin effect depends on the rapid release by the gut of several hormonal factors (incretins) in response to the ingestion of nutrients. Among these factors, gastrointestinal peptide (GIP) and GLP-1 play a major role. The latter, in particular, has been shown, under hyperglycaemic conditions, to stimulate insulin secretion, suppress glucagon release, and normalize blood glucose levels without causing hypoglycaemia [85]. But GLP-1 exerts more
actions that just stimulating insulin release in response to meal ingestion.

GLP-1 can modulate the expression of many β-cell specific genes [86] such as proinsulin, PDX-1, GLUT-2, and glucokinase. Some of these genes are directly involved in the regulation of β-cell mass, and GLP-1 can act as a growth factor on these cells and favor differentiation of ducatal cells. This is an important observation since pancreatic duct cells are believed to differentiate toward β-cell to replace the dying or malfunctioning ones. Administration of exendin-4, a GLP-1 analog, to partial pancreatoctomized animals improves glucose tolerance through increased β-cell replication, neogenesis and β-cell mass [87]. Treatment of db/db mice with exendin-4 resulted in stimulation of ductal cell differentiation and initiation of β-cell neogenesis [88].

GLP-1, however, may favor β-cell mass preservation or growth because it also exerts an exquisite inhibitory effect on apoptosis. GLP-1 infusion in ZDF rats is associated with a significant reduction in the number of apoptotic cells [89], increased islet size and proliferation rate and improvement of glucose tolerance. Exendin-4 reduces apoptosis in streptozotocin-treated animals and delays the onset of diabetes [90]. The results observed in animals have been largely confirmed by in vitro studies where GLP-1 has been shown to protect β-cells from the apoptotic insult of IL-1β, TNF-α, and interferon-γ [90] and to inhibit hydrogen peroxide-dependent apoptosis [91]. The anti-apoptotic effect is mediated by increased expression of anti-apoptotic protein bcl-2 and bcl-xL, and activation of the cAMP and PI3-kinase-dependent signaling pathway. More recently, Farilla et al [92] have confirmed this anti-apoptotic effect of GLP-1 in human pancreatic islets (Fig. 3). In these islets, GLP-1 was able to delay the morphological changes accompanying maintenance in culture of islets, increase anti-apoptotic protein bcl-2 expression, and down-regulate intracellular concentration of active caspase-3. Interestingly, maintenance of islet architecture was associated with significant improvement in β-cell function. The finding was corroborated by higher islet insulin content and greater glucose-dependent insulin secretion. In light of these experimental results, specific studies are needed to determine to what extent they can be translated to human diseased conditions.

10. Conclusions

In the light of the existing data, it is plausible that development pancreatic β-cell dysfunction is central to development and progression of type 2 diabetes. Both decreased β-cell mass and altered β-cell function contribute to the defective insulin release typical of the disease. Unraveling the underlying mechanisms may be a difficult task, but several genetic and acquired factors have been identified, together with some of the molecular pathways possibly involved. Direct studies with isolated islets show that several of the deleterious effects due to acquired factors can be prevented. More importantly, evidence is emerging that some of the functional and molecular defects of type 2 diabetes β-cells can be corrected. Proliferation, neogenesis, and apoptosis in the islets of Langerhans form the basis of islet cell mass regulation and contribute to pancreatic adaptation in the initial stages of diabetes mellitus. Despite some similarities to the regulation of other endocrine pathways, the islet seems to be a more complex environment with incomplete understanding of the complex regulation of proliferation, neogenesis, and apoptosis is incomplete.

Identification of the factors conferring susceptibility (mainly genetic) and those that may accelerate such process (mainly environmental and metabolic) represents the way to elaborate therapeutic strategies capable at slowing if not arresting β-cell loss, thus contributing to maintenance of glycaemic control and reduction of the risk of developing long-term diabetic complications. Though difficult the impression is that we may be a little bit closer to a solution.

A new therapeutic era has been opened by the incretin concept. GLP-1 has been identified as a powerful insulinotropic hormone that is secreted poorly in type 2 diabetic patients. Introduction of GLP-1 analogs and molecules that inhibit DPP-IV (the enzyme responsible for rapid deactivation of GLP-1) thereby enhancing he half-life and incretin effects of GLP-1 has created much expectation in term of β-cell preservation. In vitro and animal data have demonstrated that GLP-1 can increase β-cell mass by stimulating islet cell neogenesis and by inhibiting apoptosis [85]. An ability to stimulate β-cell replication in vitro followed by up regulated expression of the transcription factor PDX-1 (which is involved in pancreatic development, islet cell function, regulation of insulin gene expression, and the increase of β-cell mass in animal models) has also been ascribed to GLP-1 [87-89]. The ability of GLP-1 and related compounds to recruit β-cells into a secretory mode, to up regulate the glucose-sensing genes of the β-cell, and to induce β-cell differentiation and neogenesis [91,92] suggests potential to reverse diabetes-associated defects in the failing β-cell [93,
It is because of this expectation that we desperately need well designed clinical trials.

**Conflicts of interest:** S. Del Prato: Advisory pannels (Novartis Pharmaceuticals, Merck & Co, Roche Pharmaceuticals, Roche Diagnostics Pharmaceutical, Pfizer Inc, Eli Lilly & Co, Amylin Pharmaceuticals Inc, Mannkind Corporation, Takeda Pharmaceuticals, Boheringer Ingelheim); Research support (Merck & Co, Pfizer Inc, Eli Lilly & Co, Sanofi Aventis); Speaker Bureau (Glaxo Smith Kline, Sanofi Aventis, Novartis Pharmaceuticals).

R. Lupi: None.

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