

# $\beta$ -Cell mass plasticity in type 2 diabetes

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## Introduction

Prospective studies have shown that insulin resistance (IR) and impaired insulin secretion are independent predecessors and predictors of overt diabetes in genetically predisposed individuals [1–3]. Studies involving subjects at high risk of progression to type 2 diabetes (T2D) have shown that  $\beta$ -cell function begins to decline prior to presentation of elevated, fasting plasma glucose levels, thus predicting the subsequent development of T2D [4]. With the onset of IR, rates of both insulin biosynthesis and secretion are amplified, coupled with increases in  $\beta$ -cell mass and hypertrophy of existing  $\beta$ -cells to meet the increase in demand [5]. As the  $\beta$ -cell mass reaches its threshold to compensate, the burden of IR can no longer be overcome [6]. In those individuals with a genetic predisposition, this lack of pancreatic plasticity ultimately leads to hyperglycaemia [7].

After years of controversy regarding the primacy of either a  $\beta$ -cell defect or IR in the pathogenesis of T2D, we now recognize that various degrees of both alterations must coexist for hyperglycaemia to become manifest. While much is known about the mechanisms of IR, the causes for  $\beta$ -cell defects remain poorly understood. Still more enigmatic is the clinical relationship of the functional  $\beta$ -cell defect to actual  $\beta$ -cell mass. Vague terms such as  $\beta$ -cell exhaustion and fatigue,  $\beta$ -cell rest and survival and  $\beta$ -cell preservation provide little direction towards the effective therapeutic strategies for preserving  $\beta$ -cell function and mass.

As the incidence of T2D continues to increase at an alarming rate, it is becoming imperative that the clinical focus shift towards the preservation of  $\beta$ -cell function

and mass. Widespread efforts are being undertaken in an attempt to understand the mechanisms that regulate  $\beta$ -cell mass and to determine precisely what goes awry in T2D. Clinicians, however, are limited by the fact that there is no method for monitoring  $\beta$ -cell mass. In the following review, we discuss factors that influence  $\beta$ -cell mass and potential strategies for therapeutic intervention in the preservation of  $\beta$ -cell mass.

## Defining $\beta$ -Cell Mass Plasticity

$\beta$ -Cell mass plasticity encompasses both expansion and involution (shrinkage) of  $\beta$ -cells in response to changes in insulin demands for maintenance of glucose homeostasis throughout the lifespan [7]. The endocrine pancreas is continually remodelled [8] in a dynamic process involving both the death and regeneration of  $\beta$ -cells. Numerous genetic, metabolic and environmental factors impact this process.

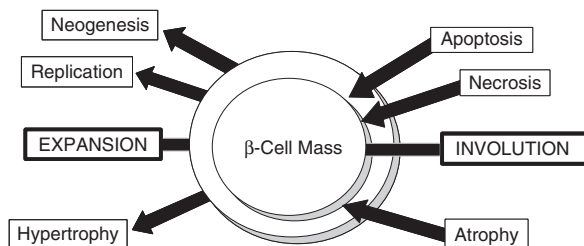
The compensatory mechanisms of a functioning  $\beta$ -cell mass, although diverse, are not mutually exclusive (figure 1). Involution and expansion of the  $\beta$ -cell mass involve changes in cell number, as well as changes in size through increases (hypertrophy) or decreases (atrophy) in volume. Furthermore, fluctuations in metabolic demand result in increased insulin secretory capacity by a complex adaptive process that includes modulation of  $\beta$ -cell mass, resetting of the  $\beta$ -cell threshold of response to stimuli and increased insulin biosynthesis and release [7,9]. Hypertrophied  $\beta$ -cells, however, are more prone to apoptosis, a genetically

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**Fig. 1** The  $\beta$ -cell mass is constantly renewed through a balance between mechanisms of expansion and involution.

programmed form of cell death, which ultimately contributes to impaired function of the  $\beta$ -cell mass [10]. Absolute numbers of  $\beta$ -cells may increase (hyperplasia) through replication and neogenesis. This increase is balanced by reduction in cell number via cell death through apoptosis or necrosis. Apoptosis, which allows for remodelling of tissue during organogenesis and throughout life, plays a critical role in modulating expansion and involution of  $\beta$ -cell mass in T2D [11,12]. During the progression from impaired glucose tolerance (IGT) to frank diabetes, increased rates of  $\beta$ -cell apoptosis account for the loss of  $\beta$ -cell mass [13].

Different mechanisms contribute towards the regeneration of  $\beta$ -cell mass. Three distinct pools of  $\beta$ -cells have been recognized: (a) a replicative pool, which responds to growth factors, stimulating existing  $\beta$ -cells to replicate within the islets through mitotic mechanisms; (2) a senescent pool, comprised of mature, functional  $\beta$ -cells that are no longer able to replicate and (3) a precursor pool, found in pancreatic ducts, from which newly differentiated  $\beta$ -cells emerge via neogenesis. The adult pancreatic duct epithelium (and possibly other pancreatic cell types as well) continues to be a source for  $\beta$ -cell neogenesis throughout the life cycle [14,15].

Specific determinants of  $\beta$ -cell hypertrophy vs. hyperplasia in response to increased demands for insulin are also poorly understood. It has been suggested that cells that maintain their replicative ability might dedifferentiate, transiently losing functionality as they replicate [9], resulting in the loss of the normally functioning  $\beta$ -cell phenotype. The compensatory mechanism for increased insulin demand in  $\beta$ -cells that are no longer able to divide (in the stage of terminal senescence) is hypertrophy. Although multiple factors influence  $\beta$ -cell replication, glucose appears to be the dominant influence [16]. Forced replication, however, may be counterproductive, resulting in loss of function due to dedifferentiation [17]. Conversely, stimulating differentiation of embryonic stem cells into  $\beta$ -cells can result in loss of the capacity for cell division [5]. Thus, regulation of  $\beta$ -cell mass is a

complex, genetically heterogeneous process, and the relative contributions of involution and expansion can vary to a great extent, even within the same individual, as physiological conditions change.

Although  $\beta$ -cell mass increases (slightly) throughout life [9,10,18],  $\beta$ -cell function declines with age [19]. The presence of peripheral IR in obese normoglycaemic individuals results in a  $\beta$ -cell mass increase to 150% of normal [18]. In contrast,  $\beta$ -cell mass may already be reduced up to 50% at the time of diagnosis of T2D [20]; a significant functional defect in the remaining cells, however, is required to account for the development of hyperglycaemia. A 40–60% reduction in  $\beta$ -cell mass in itself does not produce glucose homeostasis abnormalities in non-diabetic rodents [21,22]. In fact, 85–95% pancreatectomy is required to induce hyperglycaemia [23], and although these rodents become hyperglycaemic, the rate of apoptosis does not increase as in diabetes [24]. Therefore, while severe ablation of  $\beta$ -cell mass in itself *does* induce hyperglycaemia, the apoptotic component of T2D *does not* present, thus demonstrating that T2D is a complex polygenic disease that cannot be reproduced by simply reducing  $\beta$ -cell mass.

### Can We Quantify Human $\beta$ -Cell Mass Clinically?

A number of early functional markers may be useful in predicting progression to diabetes, at a time when  $\beta$ -cell preservation may still be feasible. During the progression from normal to IGT, and finally to overt diabetes,  $\beta$ -cell function decreases commensurately [25]. At the time of diagnosis of diabetes in the United Kingdom Prospective Diabetes Study (UKPDS),  $\beta$ -cell function was already reduced by 50% and progressively declined over time in a linear fashion. If linearity is assumed over the entire course of  $\beta$ -cell function loss, then it can be calculated that declines in  $\beta$ -cell function integrity may have begun as many as 12 years before diagnosis [26]. These findings support a role for pre-existing impairment in islet function that may be independent of, but exacerbated by, loss of compensatory increase in  $\beta$ -cell mass. Thus, assessment of  $\beta$ -cell mass may be important in diagnosis and treatment as well as in the consideration of novel drugs with the potential to preserve or even expand  $\beta$ -cell mass (as opposed to those that stimulate  $\beta$ -cell function).

Whereas several tests exist for the determination of insulin secretory capacity, *in vivo* measurement of  $\beta$ -cell mass in humans is currently not possible. Target-specific imaging probes have been recently developed for use in combination with nuclear imaging, which may facilitate *in vivo* assessment of  $\beta$ -cell mass [27]. Nevertheless, a better understanding of the relationship between mass

and function will be critical as we seek to refine methods for accurately measuring and monitoring β-cell mass.

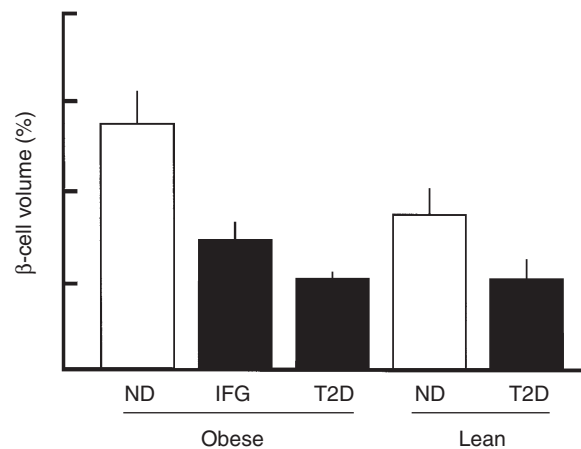
At present, we can only rely on functional surrogate measures that indirectly reflect β-cell mass. In animal studies, for instance, a correlation between acute insulin response and β-cell mass was identified [28]. Nonetheless, direct markers of insulin secretion (e.g. native insulin, C-peptide, proinsulin and split proinsulin products), as well as indirect measures of insulin secretion [e.g. HOMA-B or proinsulin:immunoreactive insulin ratios (PI:IRI)], as surrogates for β-cell mass are less than ideal. These markers may not necessarily reflect the β-cell mass, especially in the face of confounding factors, most notably IR. It is beyond the scope of this review to discuss the advantages and limitations of functional measurements as they relate to β-cell mass, but the interested reader is referred elsewhere [19].

### What Happens to β-Cell Mass in Type 2 Diabetes?

A reduction of β-cell mass has been reported in some studies of T2D populations [29,30]. Guiot *et al.* [31] found reduced β-cell mass in lean and obese patients requiring insulin therapy but not in obese T2D patients treated by diet therapy. Other studies [32,33] have shown normal or even increased β-cell mass. In a recent study [13], relative β-cell volume was found to be increased in obese vs. lean non-diabetic subjects due to increased neogenesis. Compared with people who did not have diabetes, obese individuals with impaired fasting glucose and T2D had a 40 and 63% reduction in β-cell mass, respectively, whereas lean patients with T2D had a 41% deficit (figure 2). In all cases, low rates of cell replication were observed, with no difference between diabetic and non-diabetic cases. In contrast, the rate of β-cell apoptosis was 10-fold greater in lean and threefold greater in obese patients with diabetes [13]. These results suggest that pancreatic plasticity is an important mechanism to meet the homeostatic demand for increased insulin secretion in insulin-resistant obese individuals who do not have diabetes. In addition, these findings indicate that in T2D, relative reduction of β-cell mass occurs as the result of excessive β-cell apoptosis. In the following section, we address some of the genetic and environmental factors that influence β-cell mass in the normal state and in the progression from IR to T2D.

### The Genetic Determinants of β-Cell Mass

Owing to the polygenic and complex nature of human T2D, findings from animal studies may translate poorly to



**Fig. 2** Mean relative β-cell volume in obese [non-diabetic (ND), impaired fasting glucose (IFG) and type 2 diabetes subjects (T2D)] and lean cases. Results obtained from pancreatic tissue examination from 124 autopsies: 91 obese cases (body mass index >27 kg/m<sup>2</sup>; 41 with T2D, 15 with IFG and 35 ND subjects) and 33 lean cases. Adapted from Ref. [13].

humans. Nevertheless, animal models in which diabetes results from increased rates of apoptosis have provided important clues to our understanding of the progression of the disease with regard to changes in β-cell mass. A mutation in the leptin receptor of the Zucker diabetic fatty (ZDF-*fa/fa*) rodent model leads to fat deposition in islets, lipotoxicity and β-cell decompensation. In obese Zucker fatty (ZF) rats, a fourfold increase in β-cell mass occurs to compensate for IR throughout life, thus preventing progression to diabetes. In contrast, β-cell mass increases only twofold in ZDF rats. Higher replication rates in these animals are offset by increased rates of apoptosis so that β-cell mass is only 50% of that observed in controls and is, consequently, insufficient to overcome IR and prevent diabetes [34]. This situation appears to parallel that observed in humans, who by the time of diagnosis with T2D may have already experienced up to a 50% loss of their β-cell mass [20].

### Influences of Glucotoxicity and Lipotoxicity on β-Cell Mass

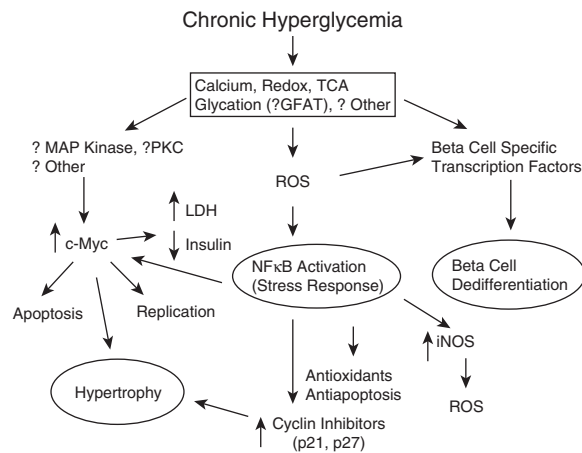
Measurements of β-cell mass are, in part, dependent on the stage of the disease. If the genetic trap for T2D is set up to a decade prior to the clinical onset of the disease, any number of environmental factors could trigger the cascade of biological events that ultimately culminate in frank T2D. What *is* known is that shortly before and after the disease becomes manifested, multiple clinical factors that might impact β-cell mass can be identified.

Both glucotoxicity and lipotoxicity engender defects in  $\beta$ -cell function and  $\beta$ -cell mass. Elevated blood glucose is a potent stimulator of insulin production through hypertrophy and hyperplasia of existing  $\beta$ -cells [7,35]. After a critical concentration threshold is achieved, however, glucose acts as a negative modulator of  $\beta$ -cell mass [36]. Prolonged exposure of  $\beta$ -cells to even mild hyperglycaemia is deleterious to the  $\beta$ -cell phenotype, with secondary effects on gene expression and reduced  $\beta$ -cell differentiation [37] – consequences likely to be underestimated in clinical practice. Hyperglycaemia leads to progressive loss of expression of a number of insulin secretory genes, a parallel reduction of key transcription factors involved in  $\beta$ -cell development and differentiation [including pancreas-duodenum homeobox factor-1 (PDX-1)] and a concomitant increase in the expression of factors involved in  $\beta$ -cell hypertrophy (including cMyc, a potent transcription factor involved in  $\beta$ -cell growth and apoptosis) [38–40] (figure 3). PDX-1 is the main transcriptional activator of glucose-regulated insulin gene expression [41] and is referred to as the master regulator of pancreas development and  $\beta$ -cell differentiation and function [42]. Although restoration of normoglycaemia can correct short-term hyperglycaemic-induced gene expression alterations [37], the point at which irreversible changes in  $\beta$ -cell function occur remains to be elucidated.

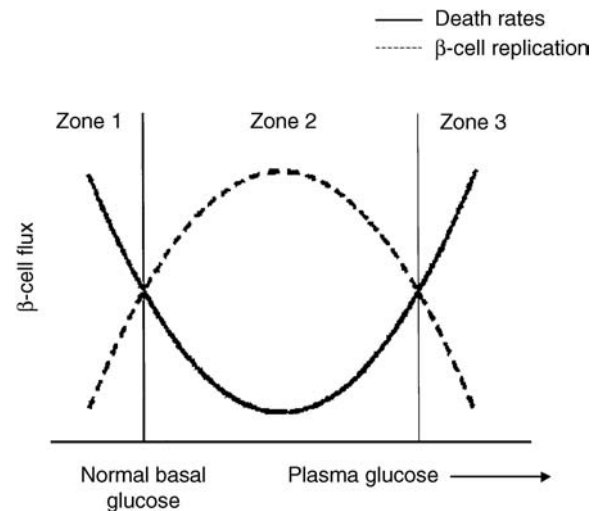
Possible links have been suggested that relate hyperglycaemia to dedifferentiation, hypertrophy and activation

of the stress response (figure 3). Although still poorly understood, many of these pathways lead to generation of reactive oxygen species (ROS) [16]. Protective stress genes (e.g. antioxidant and antiapoptotic genes) are upregulated in rat islets following pancreatectomy [24]. Nitric oxide (NO) production, however, which is known to be deleterious to  $\beta$ -cell function and survival, is also increased three-fold in these islets [40]. Finegood and Topp [43] have diagrammatically captured the variable role of glucose in  $\beta$ -cell flux (figure 4). These investigators emphasize the ability of glucose to create an imbalance between  $\beta$ -cell replication and  $\beta$ -cell death.

Long-term elevation of circulating free fatty acid (FFA) levels has been shown to diminish  $\beta$ -cell function [44–47], and accumulation of FFA in  $\beta$ -cells has been shown to be cytotoxic [48]. FFAs induce apoptosis through activation of the caspase pathway as a likely consequence of ceramide formation within the  $\beta$ -cell [49]. In animal models, the observation that leptin and thiazolidinediones (TZDs) protect  $\beta$ -cells from apoptosis further suggests that lipids are toxic to  $\beta$ -cells [7,50,51]. Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  agonists have been shown to directly protect  $\beta$ -cells from lipotoxicity in human pancreatic islets. These protective effects include prevention of FFA-induced



**Fig. 3** Hypothetical scheme about how chronic hyperglycaemia might lead to alterations of the  $\beta$ -cell phenotype in diabetes, which include  $\beta$ -cell dedifferentiation, hypertrophy and activation of stress-response genes. GFAT, fructose-6-phosphate amidotransferase; iNOS, inducible isoform of nitric oxide synthase; MAP, mitogen-activated protein; PKC, protein kinase C; ROS, reactive oxygen species; TCA, trichloroacetic acid. Reprinted with permission from Ref. [16].



**Fig. 4** Modelling of the effects of plasma-glucose concentration on the  $\beta$ -cell replication and death rates. Below normal glucose levels, the death rate exceeds the replication rate (zone 1), but as glucose levels increase, the rate of  $\beta$ -cell replication becomes greater than the rate of death (zone 2), causing  $\beta$ -cell mass to increase and the glucose level to return towards normal. However, if blood glucose levels continue to rise, the rate of  $\beta$ -cell death exceeds the rate of replication (zone 3), causing  $\beta$ -cell mass to decrease and driving the glucose level even higher. Reprinted with permission from Ref. [43].

downregulation of PPAR- $\gamma$  and an insulin mRNA expression, in addition to inhibition of glucose-stimulated insulin secretion [48,52]. Moreover, PPAR- $\gamma$  agonists have been shown to block the accumulation of islet TG that precedes  $\beta$ -cell failure, thus preserving  $\beta$ -cell function [51,53]. Such preclinical results suggest novel potential strategies for preventing lipo-apoptosis of  $\beta$ -cells including reduction of FFAs/TG in islets, inhibition of ceramide formation (and consequent NO production) and the restoration of leptin action, which limits FFA entry into potentially toxic non-oxidative metabolic pathways [51].

Recent findings indicate that impaired insulin secretion induced by hyperlipidaemia is observed only in the presence of hyperglycaemia. Normoglycaemic islets retain normal  $\beta$ -cell function under experimentally equivalent conditions [54], supporting the interdependence of glucotoxicity and lipotoxicity. It has been proposed that glucotoxicity occurs independently of lipotoxicity, whereas lipotoxicity occurs only with concomitant hyperglycaemia [55].

### The Effect of Amyloid on $\beta$ -Cell Mass

Islet-amyloid polypeptide (IAPP), or amylin, is a normal secretory product of the pancreatic  $\beta$ -cell and is colocalized with insulin in the secretory granules [56]. Islet amyloid is formed primarily by deposition of IAPP in the form of fibrils; the exact mechanism of amyloidogenesis, however, remains unknown. A potential role for IAPP in the development of IR,  $\beta$ -cell dysfunction and T2D has been examined; unfortunately, these studies have yielded conflicting and inconclusive results [57–61]. In contrast to studies that found no association between amyloid deposits and the duration of T2D [62], others have shown an association between islet amyloid deposits and apoptosis, replacement of  $\beta$ -cell mass and decline in  $\beta$ -cell function [6,7,63].

Some investigators have concluded that up to 90% of patients with T2D have amyloid deposits in their islets [43] and that the degree of amyloidosis correlates with the duration and severity of the disease [64]. The formation of amyloid polymer within the islet creates space-occupying lesions responsible for defects in both insulin secretion and absorption. Human amyloid is toxic to  $\beta$ -cells and contributes to loss of  $\beta$ -cell mass [65,66]. In animal models, islet amyloidosis shows a diffuse distribution throughout the pancreas, with progressive diminution of endocrine mass as amyloid deposit increases [67]. As amyloid deposit increases,  $\beta$ -cell mass decreases, resulting in impaired  $\beta$ -cell function and glucose intolerance.

### Effects of Oxidative Stress and Cytokines on $\beta$ -Cell Mass

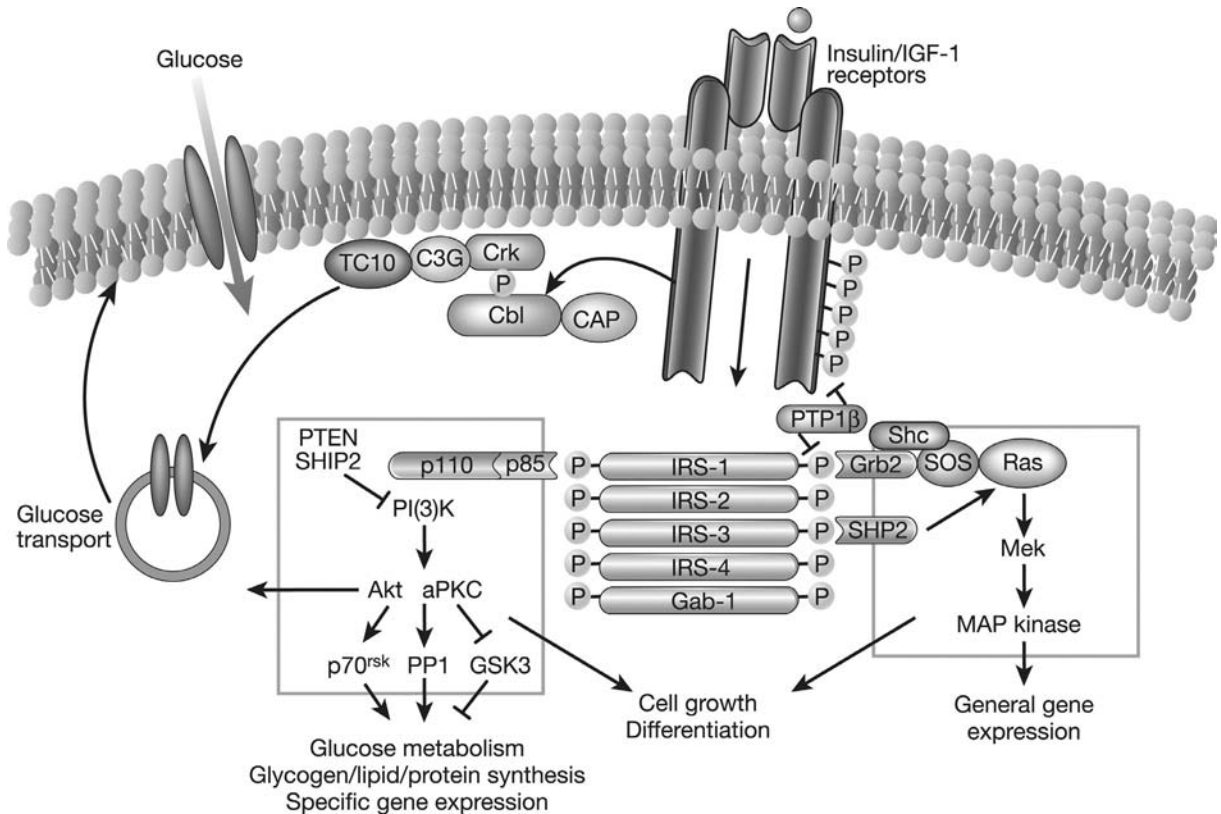
Oxidative stress generated by metabolic disturbances of diabetes has been implicated in causing diffuse tissue damage via accelerated apoptosis and a resultant reduction of  $\beta$ -cell mass [68]. Furthermore,  $\beta$ -cells with dysregulated glucose sensitivity may be more vulnerable to destruction via oxidative damage [69]. Oxidative stress from hyperglycaemia, and possibly excess FFAs, can worsen IR, impair insulin secretion and contribute to the development of long-term complications of T2D [70]. ROS are believed to play a direct role in the pathogenic effect of high glucose, possibly in concert with FFAs, by damaging DNA, protein and lipids and by activating stress-sensitive pathways that cause cellular damage [70] (figure 3) such as activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). This transcription factor is the target of hyperglycaemia, ROS and oxidative stress, playing a key role in mediating immune and inflammatory responses and apoptosis (figure 3).

Oxidative stress and ROS also activate serine kinases, whose targets include the insulin receptor and insulin receptor substrate (IRS) proteins. The activation of serine kinase signalling cascades may lead to increased phosphorylation of IRS-1 and IRS-2, ultimately decreasing the activities of downstream signalling molecules (e.g. phosphatidylinositol 3-kinase [PI(3)K]) and resulting in IR [70,71] (figure 5). Effects of oxidative stress in the insulin-signalling cascade of the  $\beta$ -cell can contribute to impairments in both insulin secretion and  $\beta$ -cell maintenance.

### How Might We Clinically Modify $\beta$ -Cell Mass Plasticity in T2D?

#### Sulfonylureas and Metformin

Sulfonylureas (SUs) and metformin, existing therapies for T2D, failed to arrest the inexorable decline of  $\beta$ -cell function over the course of the 6-year UKPDS [26], suggesting that in spite of improvement of the metabolic environment, these treatments are either ineffective in preventing  $\beta$ -cell loss or were initiated too late in the course of the disease to prevent further decline in insulin secretory function. More recently, metformin has been shown to restore  $\beta$ -cell secretory function *in vitro* [47,72], but no effects on the preservation of  $\beta$ -cell mass have been reported, and these findings remain to be confirmed in humans. Finally, SUs have been shown to trigger  $\beta$ -cell apoptosis [12], which may, over the time, contribute to reductions in both  $\beta$ -cell function and mass.



**Fig. 5** Signal transduction in insulin action. The insulin receptor is a tyrosine kinase that undergoes autophosphorylation and catalyses the phosphorylation of cellular proteins such as members of the IRS family, Shc and Cbl. Upon tyrosine phosphorylation, these proteins interact with signalling molecules through their SH2 domains, resulting in a diverse series of signalling pathways including the activation of phosphatidylinositol 3-kinase [PI(3)K] and downstream PtdIns (3,4,5) P3-dependent protein kinases, ras and the MAP kinase cascade, and Cbl/CAP and the activation of TC10. These pathways act in a concerted fashion to coordinate the regulation of vesicle trafficking, protein synthesis, enzyme activation and inactivation, and gene expression, which results in the regulation of glucose, lipid and protein metabolism. Reprinted with permission from Ref. [71].

**PPAR- $\gamma$  Agonists/Thiazolidinediones**

In addition to reducing serum levels of glucose, FFAs and TG, TZDs decrease ectopic fat deposition (intracellular lipid) in liver, skeletal muscle and in  $\beta$ -cells [73]. TZDs have been shown to: (a) reduce TG accumulation in ZDF islets, resulting in improved  $\beta$ -cell function [53]; (b) prevent loss of insulin secretion (and insulin stores) of cultured human islets exposed to high FFA concentrations [73,74]; and (c) prevent FFA-induced IR in Wistar rats [75]. These effects may be due to the ability of TZDs to prevent the suppression of PPAR- $\gamma$  expression that occurs in human islets in the presence of high levels of FFAs [73].

A TZD class effect has been demonstrated with three different TZDs (pioglitazone, rosiglitazone and troglitazone), all of which have been shown to decrease the PI:IRI ratio, suggesting an improvement in  $\beta$ -cell function [76,77]. TZDs have also been shown to stimulate and

maintain pancreatic growth in normal rats by stimulating pancreatic hyperplasia, suppressing islet fibrogenesis, preventing or even reversing age-related decline in insulin sensitivity and improving glucose metabolism [78].

Preliminary findings from the TRIPOD study (**TR**oglitazone **In** Prevention **O**f **D**iabetes) suggest that  $\beta$ -cell function may be preserved with early intervention using TZDs. Administration of troglitazone to women with prior gestational diabetes in the TRIPOD study resulted in a >50% reduction in the incidence of T2D in this high-risk population [79]. Reduction of secretory demands on  $\beta$ -cells through reversal of IR greatly reduced the risk of developing diabetes. Additionally, in accordance with the hyperbolic relationship between insulin sensitivity and secretion identified by Kahn [4], glucose tolerance was maintained despite a >60% increase in insulin sensitivity, suggesting a ‘functional resetting of  $\beta$ -cell

compensation' [80]. The patients who benefited the most from TZD treatment in these studies were those who exhibited the most residual  $\beta$ -cell activity and, thus, were able to respond to the improved insulin sensitivity with a large downregulation in  $\beta$ -cell secretion [79]. It is notable that protection from T2D persisted eight months after the completion of the study, indicating that this may be a prolonged effect, as opposed to simply masking the onset of diabetes. These findings have recently received support in the PIPOD (**P**ioglitazone **I**n **P**revention **O**f **D**iabetes), a follow-up study in which protection from diabetes observed in the TRIPOD persisted during the first year of treatment with pioglitazone [81]. Moreover, women who had been on placebo in the TRIPOD experienced reductions in glucose and insulin levels with pioglitazone treatment, indicating protection against T2D. These results support the concept that IR is either causative or contributory in  $\beta$ -cell functional decline and the eventual development of T2D.

### Insulin

Insulin therapy reduces blood glucose and lowers TG and FFA concentrations, thus reducing glucotoxicity and lipotoxicity and possibly improving  $\beta$ -cell function. Early insulin therapy in asymptomatic diabetes might restore pancreatic function and perhaps even induce remission periods [82,83]. Ilkova *et al.* [84] have reported that early intensive insulin therapy with continuous subcutaneous insulin infusion in newly diagnosed T2D patients is associated with prolonged preservation of glycaemic control (i.e. persistence of  $\beta$ -cell function).

Insulin may indirectly influence  $\beta$ -cell mass, as suggested by studies in which mice deficient in the islet insulin receptor IRS-2 developed diabetes due to a reduction in  $\beta$ -cell mass and altered  $\beta$ -cell function [85]. However, recent data indicate that insulin may affect  $\beta$ -cell growth via activation of the insulin/insulin-like growth factor-1 (IGF-1) receptor by IRS-2 [7]. IGF-1 modulates  $\beta$ -cell mass through activation of signal transduction pathways (figure 5). Signalling from this receptor, specifically, has been shown to inhibit  $\beta$ -cell death through activation of PI(3)K [86], whereas subsequent IGF-mediated activation of Akt (protein kinase B) plays a role in  $\beta$ -cell survival [87].

### Future Targets

#### Oxidative Stress and Cell Defence Mechanisms

Reduction of hyperglycaemic-induced ROS toxicity can preserve  $\beta$ -cell mass *in vivo* by suppressing apoptosis,

preserving insulin content in the islets and stimulating the expression of PDX-1 [68]. Oxidative stress inhibits IKK $\beta$ , a modulator of NF- $\kappa$ B (figure 3), the transcription factor that mediates immune and inflammatory responses and apoptosis, including the expression of several genes linked to the complications of diabetes [70]. Suppression of IKK $\beta$ -mediated proinflammatory pathways results in reversal of IR [88] and may represent a potential target in the treatment of T2D.

Defects in defence mechanisms (e.g. stress response genes) may also become pharmacologic targets. For example, the identification of a role for caspase (a protease whose inhibition blocks islet-cell death), the involvement of the ceramide pathway (resulting in free-radical-induced damage) and the regulation by Bcl-2 (a gene product with the potential to facilitate cell survival) in the proapoptotic effects on human pancreatic islets also present opportunities for specific drug targets [49].

### Incretin Mimetics

Glucagon-like peptide-1 (GLP-1) has been shown to normalize glucose tolerance in T2D patients through its potentiating effect on glucose-stimulated insulin release [89]. An ability to stimulate  $\beta$ -cell replication *in vitro* associated with upregulated expression of PDX-1 (which is involved in pancreatic development, islet-cell function and regulation of insulin gene expression) and increased  $\beta$ -cell mass in animal models has also been ascribed to GLP-1 [90–92]. Even in older, glucose-intolerant rats, GLP-1 stimulates a reversal of the age-related decline in  $\beta$ -cell function. In these animals, GLP-1 restores first-phase insulin response to glucose by sensitizing previously unresponsive  $\beta$ -cells to glucose and by increasing the amount of insulin secreted per cell [93]. GLP-1 and exendin-4 (a longer-acting agonist for the GLP-1 receptor) trigger (a) the recruitment of  $\beta$ -cells into a secretory mode, (b) the upregulation of the genes of the  $\beta$ -cell glucose-sensing machinery and (c) the induction of  $\beta$ -cell differentiation and neogenesis [90,91,94]. These effects suggest the potential for recovery of the failing  $\beta$ -cell [92,95].

### Induction of $\beta$ -Cell Rest with ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) Channel Openers

ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels couple  $\beta$ -cell metabolism to electrical activity leading to insulin secretion [96]. Animals with disrupted K<sub>ATP</sub> channels exhibit increased numbers of apoptotic  $\beta$ -cells, indicating that K<sub>ATP</sub> channels may play an important role in  $\beta$ -cell survival and differentiation [97]. This effect is likely due to the relief of Ca<sup>2+</sup> toxicity.

In adults with recent onset of type 1 diabetes, use of  $K_{ATP}$  channel openers has been shown to enhance insulin production, thereby exerting a protective effect *in vivo* [98]. Moreover,  $K_{ATP}$  channel openers improve glucose oxidation rate, insulin content and secretion and even counteract streptozotocin (STZ)-induced islet toxicity [99,100]. Preservation of residual  $\beta$ -cells has been observed upon the induction of  $\beta$ -cell rest with  $K_{ATP}$  channel openers and continuous insulin treatment in animal models. Near-normal C-peptide responses and insulin-positive cells have also been shown after such treatment.  $K_{ATP}$  channel openers also protect insulin-producing cells against toxic damage, especially during ongoing insulinitis, a condition often observed in human diabetes [100]. Preliminary human data suggest that  $\beta$ -cell rest represents a promising strategy for preventing the progression of type 2 and latent autoimmune diabetes in adults [101].

### The Akt Signalling Pathway

Akt (protein kinase B) is a serine/threonine kinase involved in insulin signal transduction (figure 5). Signals both upstream and downstream of Akt affect  $\beta$ -cell size and growth, suggesting that this kinase may relay those signals [102]. Overexpression of Akt leads to (a) an increased size and number of islet cells and an increased  $\beta$ -cell mass and insulin content via hypertrophy, neogenesis and proliferation; (b) protection from STZ-induced diabetes; and (c) rescue of the diabetic phenotype [103]. Expression of active Akt in  $\beta$ -cells also reverses diabetes (correcting both the islet-cell mass defect and metabolic abnormalities) in IRS-2 null mice [103]. Akt also promotes  $\beta$ -cell survival via inhibition of FFA-induced apoptosis [104]. Thus, although Akt is probably not *the* physiological mediator of  $\beta$ -cell growth, agents that modulate Akt signalling may have beneficial effects on  $\beta$ -cell mass and secretory capacity.

### $\beta$ -Cell Growth Factors

Another approach for modifying  $\beta$ -cell mass plasticity has been studied in the effort to improve outcomes of human islet transplantation. Targeted overexpression of growth factors in  $\beta$ -cells increases islet size and number and improves glycaemic control in mice that receive transplants [105].

Hepatocyte growth factor (HGF) has mitogenic and antiapoptotic properties and acts as an insulinotropic factor for  $\beta$ -cells [105]. In addition to improving *in vivo* islet mass and function, HGF also increases  $\beta$ -cell proliferation, glucose-stimulated insulin secretion and

glucose tolerance and markers of  $\beta$ -cell differentiation. Similarly, placental lactogen (PL) is one of the most potent factors known to influence  $\beta$ -cell mass plasticity by increasing  $\beta$ -cell proliferation, insulin content and insulin secretion from islets both *in vitro* and in animals [106]. *In vivo* expression of PL accelerates rates of  $\beta$ -cell proliferation (hyperplasia) and increases  $\beta$ -cell size (hypertrophy), leading to two-fold augmentation of islet mass and a 1.5-fold increase in islet-cell number [106].

Overexpression of parathyroid hormone-related protein (PTHrP) exploits a different mechanism in increasing islet-cell mass. In contrast to the enhancing effects of HGF and PL on  $\beta$ -cell proliferation rate and enlargement of  $\beta$ -cell size, PTHrP increases islet-cell mass through inhibition of  $\beta$ -cell apoptosis [107]. Resistance to the diabetogenic effects of STZ has also been observed with HGF, PL and PTHr, implying that all three growth factors exert a protective effect on  $\beta$ -cells. Use of a targeted insulin promoter allows for markedly higher expression of these growth factors in pancreatic islets compared with other tissues, suggesting that their therapeutic overexpression could be successfully targeted to  $\beta$ -cell strategies aimed at enhancing islet mass and function.

### Modulation of Insulin Receptor Action and Insulin Sensitivity

Insulin itself may serve as a trophic factor for the  $\beta$ -cell. Mice deficient in islet IRS-2 develop diabetes due to a reduction in  $\beta$ -cell mass and altered  $\beta$ -cell function [85,108]. These animals develop progressive  $\beta$ -cell failure, which exacerbates the IR already present in liver and skeletal muscle, leading to diabetes. Thus, insulin may be the product, as well as an autocrine regulator, of the  $\beta$ -cell [109]. In this IRS-2 knockout mouse, PDX-1 is able to rescue  $\beta$ -cell function [110]. Increased PDX-1 expression may be valuable in protecting the  $\beta$ -cell mass directly by increasing neogenesis and may allow for the transformation of non-insulin-secreting cells into insulin-secreting cells at sites other than the pancreas [111].

Additional components of the insulin-signalling cascade may play a role in the regulation of  $\beta$ -cell mass. Ablation of p70, a substrate of Akt in insulin signalling (figure 5), is associated with decreased  $\beta$ -cell size [108]. Other data suggest that signals that regulate insulin secretion in the IRS-1 cascade diverge downstream with signals that regulate  $\beta$ -cell growth [102]. Accili [102] speculated that insulin is the primary promoter of  $\beta$ -cell neogenesis, acting through a paracrine mechanism where insulin stimulates progenitor cells to differentiate into  $\beta$ -cells. This does not rule out the possibility that a shortage in the supply of precursor cells may

predispose to diabetes even in the presence of paracrine stimulation [102].

Several additional potential targets for preservation of  $\beta$ -cell mass may be foreseen. These include: (a) direct potentiation of the action of the insulin receptor itself, e.g. by protein tyrosine phosphatase (PTP)-1B that mediates dephosphorylation of the insulin receptor (figure 5) and (b) modulation of IR by acting on tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), leptin, adiponectin, PI(3)K, a TZD target involved in  $\beta$ -cell proliferation, apoptosis and shuttling of PDX-1 in response to glucose and insulin concentrations and dephosphorylation of its products by SH2 domain containing inositol-5-phosphatase (SHIP2) (decreases of which are involved in enhanced insulin sensitivity) (figure 5) [93,112,113]. Sustained activation of mitogenic signalling pathways such as the IRS-2-mediated signalling by IGF-1 in  $\beta$ -cells (figure 5) may also represent a viable strategy in upregulating  $\beta$ -cell mass [114].

### Conclusions and Perspectives

The ability of the  $\beta$ -cell mass to increase or decrease in size is referred to as  $\beta$ -cell mass plasticity. This feature accounts, in part, for the adaptive insulin secretory response to meet changes in metabolic needs for insulin. Another component of that adaptation involves changes in  $\beta$ -cell function including increased insulin production coupled with qualitative and quantitative changes in insulin secretion. The importance of adaptive processes in the normal metabolic environment, as well as in disease states such as starvation, obesity and diabetes, is now being recognized.

Early in the natural history of T2D,  $\beta$ -cell mass increases to adapt to IR. Ultimately, the  $\beta$ -cell mass reaches its threshold to compensate, and as insulin secretion becomes inadequate, hyperglycaemia ensues. An assault on  $\beta$ -cell mass and function occurs in T2D secondary to toxins and disease-related changes in the  $\beta$ -cell phenotype. Recent evidence demonstrates that the induction or suppression of genes by hyperglycaemia itself contributes significantly to  $\beta$ -cell dysfunction [37].

While we have been able to monitor qualitative and quantitative functional aspects  $\beta$ -cell secretory capacity throughout the natural history of T2D, our knowledge regarding the changes in  $\beta$ -cell mass is mainly deductive. The reason for the lack of hard evidence is twofold: pancreatic biopsy is not possible in humans and a reproducible, non-invasive method does not exist for measuring  $\beta$ -cell mass *in vivo*. Current speculation about the behaviour of  $\beta$ -cell mass in human diabetes has been largely derived from conflicting and scanty human autopsy data and from animal models. Nevertheless,

the preservation of  $\beta$ -cell mass represents a valuable therapeutic target in treating, if not preventing, T2D.

To date, therapeutic approaches for protecting the  $\beta$ -cell and insulin secretion have been indirect, having focused on reducing blood glucose in an attempt to improve the metabolic environment (lipotoxicity and glucotoxicity) responsible for accelerating the loss of  $\beta$ -cell function after the onset of diabetes. The recent identification of specific  $\beta$ -cell transcription factors (e.g. PDX-1),  $\beta$ -cell toxins (e.g. cytokines and FFAs) and the negative effect of IR has prompted renewed interest in discovering therapies that would preserve the  $\beta$ -cell mass or even promote growth of the  $\beta$ -cell mass. Newer agents such as TZDs have been shown to positively impact  $\beta$ -cell mass in animal models, possibly secondary to their ability to reduce IR, hyperglycaemia, cytokines and FFAs. The question, however, remains whether the same effect occurs in humans.

The identification of newer targets responsible for  $\beta$ -cell apoptosis, neogenesis and proliferation and development of specific compounds (e.g. GLP-1 and exendin-4) aimed at these targets offers potential for future clinical therapies that will impact the pathophysiology of T2D in its earlier stages when  $\beta$ -cell mass may still be close to normal and functionally intact.

Several requirements, however, remain to be fulfilled in order for  $\beta$ -cell mass to become a legitimate target for therapy in the future. First, a simple, non-invasive method for measuring  $\beta$ -cell mass is necessary. Second, the influence of disease states on  $\beta$ -cell mass will need to be confirmed. In parallel, the development of new therapeutic agents that target factors controlling  $\beta$ -cell mass (e.g. cytokines and gene transcription factors) must occur. Ultimately, clinicians will need to understand the consequences of changes in both  $\beta$ -cell mass and  $\beta$ -cell function prior to and within the progression of diabetes in order to take advantage of and apply specific targeted therapeutic measures before irreversible destruction occurs.

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