Type 2 diabetes: The pathologic basis of reversible beta-cell dysfunction

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Abstract

The reversible nature of early type 2 diabetes has been demonstrated in in vivo human studies. The associated pathophysiologic studies have demonstrated return to normal of the elevated intra-pancreatic triglyceride content simultaneously with restoration of first phase insulin secretion. Recent studies of beta-cell biology both in vivo and in vitro have established that the beta-cell loses differentiated characteristics including glucose-mediated insulin secretion under metabolic stress. Critically, the beta-cell de-differentiation produced by long term excess nutrient supply is reversible. However, in type 2 diabetes of duration greater than 10 years the cellular changes appear to pass a point of no return. This review summarizes the evidence that early type 2 diabetes can be regarded as a reversible beta-cell response to chronic positive calorie balance.
Until recently, the pathophysiology of type 2 diabetes was believed to be characterised by progressive, irreversible loss of pancreatic insulin secretion [1] mediated by apoptosis of pancreatic beta-cells [2]. Now that weight loss has been shown to bring about restoration of beta-cell function and reversal of diabetes, fundamental reappraisal of the mechanisms underlying beta-cell dysfunction is required. The recent in vivo and in vitro studies will be reviewed, together with the evidence that the underlying, potentially reversible, beta-cell failure is related to dedifferentiation rather than beta cell death.

**Etiological drivers of type 2 diabetes**

Population data demonstrate major increases or decreases in incidence of type 2 diabetes secondary to food excess or scarcity. This was documented in the UK during the first and second World Wars, and in Cuba in 1990-96, with an associated sharp fall in incidence and prevalence of type 2 diabetes [3; 4]. Perhaps the clearest evidence for the impact of positive energy balance in those with a predisposing genotype is provided by the Pima Indians, who had neither excess obesity nor excess diabetes when living as subsistence farmers [5; 6]. In 1940, diabetes prevalence was similar to that of the general US population [7]. Thereafter, with cessation of an agricultural lifestyle together with food oversupply, there was a dramatic increase in rates of obesity, and the prevalence of type 2 diabetes in adult Pima Indians rose to 38% [8]. There was only a modest rise in prevalence in ethnically identical Pima Indians living in Mexico under nutritional conditions which limit adult weight gain [8].

Type 2 diabetes is commonly said to be a consequence of obesity. However, in the 1970s, when the average weight of the UK population was considerably less than at present, the
Whitehall study showed only a small association between obesity and type 2 diabetes [9]. At that time it was considered that there was no major effect of obesity on the development of common Type 2 diabetes [9-11]. Although this may seem surprising from a present day perspective, the risk of type 2 diabetes rises most steeply at very high BMI’s and in 1980 only 7% of the population had a BMI >30kg/m^2 [12]. The effect of high BMI upon development of diabetes was simply not detectable. While this association with obesity is now apparent, it has not generally been recognised that diabetes risk rises steadily throughout the population weight distribution. The Nurses’ Health Study showed a four-fold increase in type 2 diabetes prevalence in women with BMI 23-25 compared to those with BMI less than 22kg/m^2 [13]. In the UK Prospective Diabetes Study, which recruited between 1977 and 1991, 36% of newly diagnosed individuals had a BMI less than 25 kg/m^2 [14]. Conversely, 72% of people with BMI >40kg/m^2 do not have diabetes [15].

The individual susceptibility to type 2 diabetes which clusters in families must be considered. The condition does not occur unless beta-cell function is no longer sufficient to overcome insulin resistance [16]. Genome wide association studies (GWAS) indicate that the vast majority of type 2 diabetes-associated genes are likely to have a beta-cell specific role, relating to impaired ability to cope with metabolic stress [17]. Although the molecular mechanisms remain to be defined, it is likely that a range of susceptibility to beta-cell damage exists with differences between individuals.
The pathologic basis of disease progression

At diagnosis of type 2 diabetes, beta-cell function is typically reduced to 50% of normal by HOMA modelling and to a greater extent on dynamic testing [1; 18]. Despite initial impact of diet and oral glucose lowering therapy, observational studies have shown that disease progression is associated with inexorably declining beta-cell function and progression to insulin commencement with relatively minor changes in underlying insulin resistance. Such observations have been made in the context of continuing weight gain [19].

Metabolic studies have enabled elucidation of dynamic changes in beta-cell function and insulin resistance over time. In contrast, absence of imaging modalities with sufficient resolution to image the beta-cell in situ has prevented determination of whether these changes are caused by dysfunction or true loss of beta-cell mass at a cellular level [20]. Moreover, satisfactory circulating markers of beta-cell death or proliferation are currently lacking [21].

Post mortem pancreatic pathology studies, based on presence of staining for insulin containing cells, have suggested that beta-cell mass is significantly reduced in type 2 diabetes in comparison to age, sex and BMI-matched non-diabetic controls [2]. While it is accepted that increased apoptosis plays a role in decreased beta-cell mass over time, pancreatic pathology findings in a large cohort of European subjects indicate that apoptosis alone is insufficient to explain the profound islet dysfunction in established type 2 diabetes [22]. Other factors must contribute to the described decrease in cells which stain positive for insulin in the pancreatic islets.
Glucolipotoxicity

In diabetes-prone rodent models of type 2 diabetes, the beta-cell fails during overfeeding in those with the genetic predisposition, and this genetic susceptibility to lipid availability is reflected in studies on isolated islets [23]. When fatty acid concentrations are elevated in vitro, lipid synthesis and storage within the beta-cell is favored and chronic exposure of the beta-cell to fatty acid excess directly impairs glucose-stimulated insulin secretion (Figure 1)[24-26]. Prolonged exposure to elevated levels of fatty acids in vitro directly results in beta-cell stress and dysfunction [27].

Exposure of the INS1 beta-cell line to oleic acid brings about storage in intracytoplasmic vacuoles, whereas the saturated fatty acid palmitate induces expansion of the endoplasmic reticulum (Figure 1A&B) [27]. This is known to be associated with markers of endoplasmic reticulum stress, which are typically elevated in human beta-cells from individuals with type 2 diabetes [28-30]. The more physiologic exposure to mixed saturated and unsaturated fatty acids decreases insulin secretion and subsequent removal of fatty acid from the medium allows return of insulin secretion over 24 hours (Figure 1C&D) [27]. Human islets also take up fatty acids avidly and incubation in 0.33 mmol/l palmitate leads to a very large increase in islet triglyceride content, associated with markedly impaired function (Figure 1E&F)[25]. Once hyperglycemia occurs, the concomitant elevated glucose is likely to compound the metabolic insult [31].

Despite the growing body of in vitro and in vivo animal data [31] in support of an important role of glucolipotoxicity in type 2 diabetes pathogenesis and progression, human studies have proved more challenging. Hyperglycemia reversibly impairs insulin secretion in vivo [32; 33]. The combination of hyperglycemia and raised plasma free fatty acids has an
additive effect [34]. Prolonged Intralipid infusion severely impairs beta-cell function in subjects predisposed to develop type 2 diabetes [35]. Removal of excess lipid from the pancreas (by decreased supply in the face of continuing oxidation for energy needs) at the same time as decreasing plasma VLDL-triglyceride allows return of normal insulin secretion in early type 2 diabetes [36; 37]. The removable excess of intra-pancreatic triglyceride by weight loss has been shown to be specific to type 2 diabetes in a recent study using optimised pancreatic magnetic resonance imaging [38].

**Beta-cell dedifferentiation**

Loss of fully differentiated phenotype is a recently recognised potential mechanism underlying loss of beta-cell function in type 2 diabetes [39-43]. The metabolic stress of chronic nutrient oversupply can lead to reduced expression or nuclear activity of key beta-cell transcription factors including Pdx1, Nkx6.1 and MafA [39-41]. This results in loss of critical end-differentiated genes including insulin itself in parallel with induction of ‘disallowed genes’ including lactate dehydrogenase and hexokinase [44]. Beta-cell dysfunction ensues due to a combination of decreased insulin biosynthesis and loss of physiological nutrient-secretion coupling. This has been demonstrated in a number of models of glucotoxicity including partial pancreatectomy with changes largely prevented by maintenance of normoglycemia [45]; and in beta-cells in vitro following chronic palmitate exposure [46].

The Accili group have proposed an important role of the FoxO transcription factors in type 2 diabetes pathogenesis. Initially, enhanced FoxO1 nuclear translocation appears to maintain the activation state of a subset of beta-cell transcription factors, including MafA, preserving
glucose oxidation and suppressing fatty acid oxidation thus limiting mitochondrial stress [47].
Through this mechanism, FoxO1 is able to orchestrate a compensatory response aimed at preserving beta-cell function under metabolic stress (Figure 2). Establishment of chronic hyperglycemia, however, leads to FoxO1 degradation and beta-cell decompensation with impaired capacity for glucose oxidation and increased ability to oxidise palmitate. This has been described as ‘metabolic inflexibility’ with generation of toxic products including peroxides and impaired insulin secretion [47].

Lineage tracing studies in mice with beta-cell specific deletion of FoxO1 exposed to metabolic stressors, including ageing and multiple pregnancies, demonstrated that loss of beta-cell mass was due to dedifferentiation as opposed to death [41]. Whilst maintaining expression of the endocrine marker chromogranin A (ChgA), expression of insulin was lost in parallel with a number of key beta-cell transcription factors (PDX1, Nkx6.1 and MafA). Furthermore, lineage traced dedifferentiated cells (also referred to as ‘empty’ cells) expressed a number of genes not normally associated with adult beta-cells, including mesenchymal markers (vimentin) and pancreatic progenitor markers such as neurogenin 3 (Figure 2). Relevance to type 2 diabetes was evidenced by the demonstration that progressive loss of FoxO1 was associated with a large number of ChgA+/insulin- cells in rodent models of type 2 diabetes. Given the inability to directly assess cellular pathologies in people with diabetes, translation of these studies to an understanding of human pathophysiology has been challenging. Although beta-cells cannot be genetically labelled for in situ lineage tracing studies in humans, rodent models [39; 41; 48] have enabled identification of specific transitional phenotypes which can be stained for in pathological pancreatic samples. These can help determine the characteristics and fate of human beta-cells during the course of clinical diabetes.
Supported by detailed quantitative human pathology analysis, it is now established that impaired beta-cell function in type 2 diabetes cannot be accounted for by increased apoptosis alone [22; 49]. Recent \textit{post mortem} studies demonstrate that beta-cell dysfunction in type 2 diabetes may be associated with degranulation and alterations in beta-cell phenotype. Expression of key beta-cell transcription factors, including PDX1 and MafA, is markedly reduced in type 2 diabetes, suggesting that dedifferentiation (defined by loss of canonical beta-cell markers) may contribute to impaired beta-cell function [50; 51]. Furthermore, expression of disallowed genes, including vimentin, has been detected in beta-cells from patients with type 2 diabetes, providing support for the observations in rodents made by Talchai \textit{et al} [41]. In a recent pancreas pathology study, ‘empty’ beta-cells were assessed by co-immunofluorescent staining for the endocrine secretory granule marker, synaptophysin, and all endocrine hormones (insulin, glucagon, somatostatin and pancreatic polypeptide) [52] as described in \textit{db/db} and GIRKO mice [53]. Through employment of this approach and normalisation to beta-cell number, the study determined that there was a significant increase in the number of ‘empty’ or dedifferentiated endocrine cells in people with type 2 diabetes in comparison to non-diabetic controls (31.0 vs. 8.7%). Employing a similar approach, Butler \textit{et al} determined that although altered beta-cell phenotypes were evident, this did not account for the beta-cell deficit in type 2 diabetes [54]. However, expression of beta-cell transcription factors was not assessed within this study, thus limiting complete determination of beta-cell (de)differentiation status. In contrast, Cinti \textit{et al} were able to demonstrate that a number of beta-cells displayed altered subcellular localisation of Nkx6.1 and MafA, with expression almost exclusively within the cytoplasm [52]. These findings are consistent with work by Spijker \textit{et al} and indicate a functional impairment (due to cellular localisation) of these key transcription factors in human diabetes [40]. The extent to which such alterations contribute
to beta-cell dysfunction remains to be determined. However, further work exploring the association between the stage of dedifferentiation and functionality is likely to yield key mechanistic insights.

Studies exploring beta-cell dedifferentiation as a mechanism underlying dysfunction have, to date, been unable to elucidate the specific underlying signalling pathways. For example, it is not possible to determine separately the impact of hyperglycemia and dyslipidemia on beta-cell (de)differentiation status in db/db mice and pathological samples from human donors with diabetes. Recent studies have sought to explore the effects of individual stresses. For example, in a transgenic mouse model with an activating $K_{ATP}$ channel mutation of beta-cells, nutrient-stimulated insulin secretion was prevented [39]. Hyperglycemia in this model was associated with beta-cell dedifferentiation, characterised by the loss of end-differentiated beta-cell markers (Pdx1, MafA). In an ex vivo human islet lipotoxicity model, incubation with palmitate led to beta-cell dysfunction associated with loss end-differentiated phenotype evidenced by reduced levels of key beta-cell transcription factors [46].

Hyperglucagonemia in type 2 diabetes

Type 2 diabetes has long been recognised to be a dual hormonal disorder with stimulation of hepatic glucose production by aberrant post-prandial hyperglucagonemia playing an important role in overall hyperglycemia [55; 56]. It remains unknown whether this state of relative glucagon over-secretion is driven by increased alpha-cell mass or simply increased function. Henquin et al observed no increases in alpha-cell mass in patients with type 2 diabetes, concluding that relative hyperglucagonemia was likely being driven by reduced
beta-cell mass and subsequent alterations in the inhibitory actions beta-cells exert on alpha cells [57]. In contrast, Yoon et al observed that reduced beta-cell mass was associated with expansion of the alpha-cell mass in a different cohort of patients with type 2 diabetes [58]. Although it was proposed that increased alpha-cell mass may be a driver of hyperglucagonemia, a role for reduced beta-cell paracrine signalling cannot be excluded in this study.

Given the limitations such cross sectional human studies pose, definitive evidence for changes in alpha-cell mass have largely been provided by rodent studies, with recent evidence proposing a potential role for beta-to alpha-cell conversion as a mechanism underlying this phenomenon [39; 53]. Through employment of the Fox01 ablation model, Talchai et al were able to demonstrate that, following metabolic stress, an increase in plasma and pancreatic glucagon was associated with beta-to-alpha cell conversion, as determined by glucagon expression in beta-derived cells [41]. In support of this, and a role for hyperglycaemia in these plasticity events, Brereton et al describe beta-to-alpha conversion in the KATP transgenic mouse model [39]. Although describing similar fate switching events, this study observed a number of bi-hormonal cells (insulin/glucagon co-expressing), indicating a direct conversion. In contrast, Talchai et al observe that expression of glucagon in beta-derived-cells only occurs once insulin is lost, with no co-expression evident. These observations may possibly be explained by the differences in each of the transgenic models used, and subsequent variations in the nature and timing of beta-cell stress. What remains consistent, however, is that both studies provide evidence for a shift in endocrine phenotype following loss of key beta-cell transcription factors.
Beta-cell dedifferentiation, characterised by the loss of beta-cell specific markers including Pdx1, Nkx6.1 and MafA, may enable conversion towards other pancreatic endocrine phenotypes which are less susceptible to nutrient-induced mitochondrial and endoplasmic reticulum stress [59]. This raises the possibility that beta-cell identity may be fragile and that metabolic stress eliminates factors which normally repress non-beta-cell identities. In support of this, deletion of Pdx1 in adult rodents leads to severe hyperglycemia in tandem with ultrastructural and physiological alpha-cell characteristics in a large fraction of the beta-cells [48]. These data are in line with beta-to-alpha-cell fate-switching events following loss of beta-cell specific transcription factors subsequent to chronic hyperglycemia [39]. Collectively, these studies indicate that alpha-cell and potentially other endocrine cell phenotypes may be ‘default’ lineages and that loss of critical beta-cell factors which suppress non-beta-cell related genes, including glucagon, during type 2 diabetes pathogenesis may lead to a shift in endocrine phenotype from beta-to alpha-cell [39; 41; 48]. In light of recent data from Marroqui et al demonstrating that alpha-cells are more resistant to metabolic stress, it is possible that beta-alpha-cell transition during diabetes may be a defence mechanism to maintain cellular mass [59]. As reversal of type 2 diabetes is associated with fall to normal of fasting plasma glucagon levels at the same time as return of normal beta cell function the in vivo human studies may be reflecting redifferentiation of beta-cells. No change in plasma glucagon is seen in people with normal glucose tolerance during similar weight loss [60].

In support of earlier work by White et al [43], a recent study observed an 8-fold increased frequency of insulin positive cells co-expressing glucagon in tissue obtained from patients with
type 2 diabetes, indicating such beta-cell plasticity occurs in human diabetes [40]. It was determined that half the bi-hormonal (insulin+/glucagon+) cells were negative for Nkx6.1. Furthermore, cytoplasmic localisation of Nkx6.1 was observed, a phenotype not evident in patients without diabetes. This was confirmed by the demonstration that a number of cells with cytoplasmic Nkx6.1 expression co-expressed glucagon [52], indicating a role for Nkx6.1 in repressing the alpha-cell programme, as described in vitro [61]. Nkx6.1+/glucagon+/insulin- cells were also identified, indicating that factors other than Nkx6.1 are critical to these phenotypic transitions [40].

Given the nature of these cross sectional studies, interpretation must be cautious. For example, it cannot be ruled out that bi-hormonal cells reflect beta-cell neogenesis. However, given that beta-cell neogenesis has been described as originating close to and within ducts [2, 62], and associated bi-hormonal cells were localised to single and small clusters of beta-cells rather than within established islets [62], this appears unlikely. Also, Nkx6.1 is mis-localised (cytoplasm) in glucagon positive cells [52]. Although these post-mortem studies cannot provide definitive evidence, a contributory role for beta-to alpha-cell conversion in hyperglucagonemia in human diabetes is supported (Figure 2).

Lessons on beta-cell plasticity from reversing type 2 diabetes

The clearest data on the time course of recovery of beta-cell function following calorie restriction in type 2 diabetes have been provided by the Counterpoint study. In this,
observations were made at 1, 4 and 8 weeks after commencing a very low calorie diet [36]. After one week there was no improvement in the first phase insulin response to a stepped insulin secretion test with arginine (SISTA). This is especially significant as fasting plasma glucose had already normalised as a consequence of the very rapid return of normal hepatic insulin sensitivity. Hence the glucotoxicity component had been removed, and could no longer be exerting a major effect in suppressing first phase insulin response. The effect of raised glucose concentrations in inhibiting insulin secretion is known to be rapidly induced and removed [32]. Similarly, other suggested mechanisms of stress-induced beta-cell dysfunction should be rapidly reversible. For instance, the apparent mitochondrial dysfunction of type 2 diabetes is seen only when fasting plasma glucose is above 8 mmol/l [63] and is rapidly corrected by suppression of plasma fatty acid levels [64]. Reversal of oxidative stress and endoplasmic reticulum stress is also associated with rapid restoration of normal beta-cell function [27; 65].

By 4 weeks into the Counterpoint study a first phase insulin response could be seen in the group as a whole, and by 8 weeks this was well within the normal range and significantly improved from baseline. The slow normalisation of first phase insulin response over 8 weeks was mirrored by a slow normalisation of total intra-pancreatic fat [36]. Over this extended time course, the intra-pancreatic triglyceride concentration gradually declined to the same level as in non-diabetic control subjects [36]. The parallel time courses of the fall in excess triglyceride within the pancreas and the recovery of beta-cell function are suggestive but not conclusive of cause and effect.
It had to be considered whether the fall in intra-pancreatic triglyceride content could merely reflect the very considerable weight loss and not be related to restoration of beta-cell function. In order to determine whether weight loss also brought about a fall in intra-pancreatic triglyceride content in normoglycemic individuals or whether it was specific to type 2 diabetes, a further clinical study was conducted. The intra-pancreatic triglyceride content in a group of people about to undergo weight loss by gastric bypass surgery was quantified both before and 8 weeks after surgery [38]. Approximately 13% weight loss occurred both in those with normal glucose tolerance and those with type 2 diabetes. The former showed no change in intra-pancreatic triglyceride, whereas those with type 2 diabetes had higher levels at baseline which fell to normal. At the same time, first phase insulin response was restored to normal. This study demonstrates that there is an increased pool of triglyceride within the pancreas in people with type 2 diabetes and that substantial weight loss is associated with clearance of this triglyceride excess. As discussed above, exposure of beta-cells to excess saturated fatty acid causes avid uptake and decreases insulin secretory response to a change in glucose concentration. It appears possible that the gradual clearance of excess triglyceride from the pancreas might be causally linked with the recovery of insulin secretion. Quantitative comparison with measurements on isolated islets in vitro is not currently feasible.

Weight loss of 15 kg in individuals with type 2 diabetes leaves many people still in the obese category. It has to be considered that fat removed from liver and pancreas by short term hypocaloric dieting might gradually be replaced from the remaining excess in subcutaneous and visceral depots. If so, follow up might be hypothesised to reveal re-accumulation of intra-pancreatic fat with or without decline in beta-cell function. This has been examined by
six months follow up after acute weight loss of people with type 2 diabetes [37]. The group which achieved post-weight loss fasting plasma glucose of <7 mmol/l demonstrated intra-pancreatic triglyceride content falling to normal levels. Critically, weight remained stable over 6 months. First phase insulin response became and remained normal in this group. There was no reaccumulation of fat in either pancreas of liver even though mean BMI was 30kg/m² [37].

Return to normal blood glucose control after weight loss is strongly related to duration of type 2 diabetes. Whereas 87% of a short-duration group (<4 years) achieved non-diabetic fasting plasma glucose levels immediately after acute weight loss, only 50% of a long-duration group (8-23 years) did so [66]. In an audit of outcome following bariatric surgery, HbA1c of <43mmol/mol (6.1%) was achieved by 62% and 26% respectively in those with duration of diabetes <4 or >8 years respectively [60], reflecting previous observations [67]. The Scandinavian Obesity Study also confirmed the importance of duration of diabetes in rates of reversal of diabetes at 2 years [68]. As the duration of diabetes increases, it appears that a point of no return is passed with progression to fully differentiated alternative endocrine lineages (either directly or following dedifferentiation) and/or irreversible apoptosis (Figure 2).

Redifferentiation provides an attractive potential underlying mechanism for recovery of beta-cell function following reduction in islet fat content over the time course observed in vivo in humans. It can be hypothesised that this time course is too slow for reversal of acute stress-induced dysfunction and too fast for beta-cell neogenesis. The dedifferentiated state
could be considered as providing a ‘hideaway’ until the metabolic insult subsides, offering
an opportunity for restoration of end-differentiated state following alleviation of metabolic
stresses. This ‘therapeutic window’ appears limited given the failure to rescue beta-cell
function in subjects with long standing diabetes [60]. It indicates the potential progressive
nature of dedifferentiation, with a point of no return in (de)differentiation status and/or
apoptosis and subsequent irreversibility of loss of function/mass. An association may be
postulated between the extent of these abnormalities, stage of diabetes and potential for
reversal through beta-cell re-differentiation (Figure 2).

The potential for re-differentiation as a mechanism underlying restoration of beta-cell
function in type 2 diabetes is illustrated by restoration of beta cell function by intensive
insulin therapy in a rodent model of hyperglycemia [42]. This restoration in beta-cell
function was associated with beta-cell re-differentiation, characterised by increased levels
of mature beta-cell markers including insulin, Pdx1 and MafA. These observations support
the proposition that, following removal of hyperglycemia and change in lipid metabolism
achieved by intensive insulin therapy, beta-cell re-differentiation can occur and contribute
to diabetes reversal. These studies were extended to demonstrate a restoration in
sulfonylurea-sensitive insulin secretion, similar to that observed in patients with type 2
diabetes following intensive insulin therapy.

It is striking that strategies targeting restored pancreatic insulin secretion directly have not
been shown to be disease-modifying in terms of restoring functional beta-cell mass,
although the ADOPT study demonstrated greater preservation of beta-cell function with the insulin sensitiser rosiglitazone compared to sulfonylurea secretagogue therapy. Use of exenatide over 3 years caused considerable weight loss and brought about some return of beta cell function [69]. Overall it appears that decreased beta-cell exposure to nutrient excess facilitates reversal of acute dysfunction and adaptive dedifferentiation (45). There is a need for specific therapies targeted towards reversal of beta-cell dedifferentiation [70-72]. The major question of how function might be returned after apparent end stage dedifferentiation requires to be addressed.

Identification is underway of candidate pathways that could be targeted to reverse beta-cell dedifferentiation associated dysfunction [73]. A small molecule inhibitor of the TGF-beta receptor (Alk5) was shown to be capable of reversing beta-cell dedifferentiation in islets isolated from mice with extreme diabetes, utilising Urocortin 3 as a marker for mature beta-cells. Potential translation to human disease was indicated by incubation of isolated human islets with ALK5 inhibitor, bringing about increased expression levels of a number of mature beta-cell markers including insulin, Pdx1 and MafA. However, administration of ALK5 inhibitor to diabetic animals failed to improve glycaemic control, causing an overall deterioration in health. This highlights the requirement for development of pathway-specific therapeutics to restore mature, functional beta-cells if a successful pharmacological approach to controlling type 2 diabetes is to be developed.
A unifying hypothesis

The 2008 Twin Cycle Hypothesis could potentially allow synthesis of the *in vivo* and *in vitro* observations [36-38]. The hypothesis postulated that chronic positive calorie balance, in the presence of pre-existing peripheral insulin resistance and hence hyperinsulinemia, would bring about steady accretion of intra-hepatic triglyceride and initiate linked vicious cycles (Figure 3) [74]. Whilst the mechanisms underlying the liver cycle are well established, the recent *in vitro* work on beta-cell dedifferentiation provides an attractive potential explanation for the operation of the pancreas cycle [74]. The increased plasma very low density lipoprotein-triglyceride of type 2 diabetes is postulated to bring about fat accumulation and beta-cell stress such that plasma glucose remains elevated for longer after meals. The consequent effects of over-supply of saturated fatty acids on beta-cell dedifferentiation could cause gradually decreasing ability to mount an acute insulin response to eating. The liver and pancreas cycles mutually interact and reinforce each other.

At a critical point, the beta cell will fail, resulting in type 2 diabetes. All of these predictions of the 2008 hypothesis have now been observed *in vivo* [36-38].

Although most people who develop type 2 diabetes are overweight or obese by BMI criteria the condition is not uncommon in those within the normal range (36% in the UKPDS population). Direct observational data suggests that a threshold effect operates, whereby ectopic fat accumulation only occurs when an individual’s capacity to store fat safely in the subcutaneous compartment is exceeded – the Personal Fat Threshold concept [37; 75]. BMI, which was originally developed as a population metric, may mislead when applied to the individual if their adult weight gain is within the normal range. The concept could also
explain the predisposition of some ethnic groups, notably south Asians, to develop type 2 diabetes at relatively low BMI.

The recent observations upon lack of restoration of the first phase insulin response in long duration type 2 diabetes after substantial weight loss [37] can now be explained in terms of beta cell dedifferentiation. The process of losing the specialised function to produce insulin can be postulated to reach a point beyond which removal of the initial insult of excess fatty acid is not followed by the restoration of nuclear expression of key beta-cell specific transcription factors and recovery of differentiated glucose-responsive insulin secretion (Figure 2).

Type 2 diabetes may now be seen as a potentially reversible state associated with longstanding nutrient overload in susceptible individuals. The beta-cell dysfunction and loss of end-differentiated beta-cell phenotype can be restored by substantial weight loss. After approximately 10 years, the onward march of beta-cell dedifferentiation appears likely to precipitate irreversible loss of insulin secretion - unless substantial decrease in body weight is achieved.

Author Contributions

MW, JS and RT each researched published and personal data, jointly synthesized ideas and wrote the manuscript. This review was written by the authors themselves with no sponsorship or influence of any other body. There are no conflicts of interest for any of the authors. The guarantor for this article is Roy Taylor.
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Legends to Figures

Figure 1

Interaction of fatty acids with beta cell ultrastructure and function: A: Effects of exposure to 0.33mmol/l oleate or B: 0.33mmol/l palmitate upon the ultrastructure of INS-1 cells. Original photomicrographs provided by Dr Katherine Pinnick and Anne Clark [27]. C: Exposure of mouse islets to oleate or palmitate (0.5mmol/l) for 48h significantly impaired glucose-stimulated insulin secretion (data from K. Pinnick [27]). D: Impairment in glucose-stimulated insulin secretion in mouse islets brought about by incubation for 72h in a fatty acid mixture (0.5mmol/l 1:1/oleate:palmitate) was not present following 48h exposure followed by 24h in fatty acid-free media (reproduced with permission from [27]). E: Effect of exposure of human islets to 0.33mmol/l palmitate on islet triglyceride content and F: islet glucose medicated insulin secretion. Graphs E and F are reproduced with permission from [25].

Figure 2

Schematic representation of possible stages of beta-cell fate changes associated with diabetes progression: During metabolic stress, FoxO1 translocates to the nucleus to orchestrate a compensatory response to help preserve glucose oxidation and suppress fatty acid oxidation. FoxO1 target genes include beta-cell transcription factors MafA and NeuroD, which through continued activation of insulin expression help preserve beta-cell function under metabolic stress. Continued stress leads to loss and/or cytoplasmic translocation of beta-cell transcription factors (Nkx6.1 and MafA) and reduced/lost insulin expression. Analysis of human and rodent data indicates that cells, at this stage of diabetes, can undergo significant plasticity events that involves the (re)expression of ‘disallowed genes’. At this stage, cells can be characterised by two distinct identities: i) Hormone ‘empty’ cells that may express progenitor (Ngn3 and Oct4) and mesenchymal (Vimentin) proteins and ii) transdifferentiated/bi-hormonal cells that express other endocrine hormones, including glucagon. Pre-clinical data indicate that recovery of beta-cell failure at this stage of diabetes can be achieved through redifferentiation of dedifferentiated/transdifferentiated cells, a phenomenon that has been implied clinically also following calorie restriction diet. Sustained metabolic stress may result in irreversible beta-cell failure through apoptosis (dedifferentiated cells) and/or established alpha-cell differentiation, however this has yet to be be confirmed in humans. Solid arrows indicate observed rodent and human phenotypes and broken arrows indicate potential cellular pathways that may account for beta-cell loss and/or recovery.

Figure 3

During long term excess calorie intake, especially in the presence of muscle insulin resistance, the raised plasma insulin levels will expedite chronic excess calorie storage from carbohydrate via de novo lipogenesis. This will promote storage of fat in the liver very gradually over years and liver insulin resistance, with a consequent tendency for a small
increase in plasma glucose (as shown by the Whitehall II study [76]). In turn, insulin secretion will increase to control plasma glucose. The further increased insulin levels will bring about a self-reinforcing vicious cycle. The increased liver fat will inevitably lead to an increased rate of export of VLDL triglyceride from the liver. Along with all other tissue, islets will be therefore exposed to higher rates of fatty acid supply and the exposure of pancreatic endocrine cells to fatty acids and their metabolites will increase. This is postulated to bring about endoreticulum stress in susceptible individuals, and eventually beta cell dedifferentiation, with relative inhibition of meal insulin secretion. The vicious cycles are postulated to interact over many years. At a personal threshold level, it is postulated that the beta-cells can no longer compensate, and plasma glucose levels will then rise relatively rapidly. The Figure is redrawn from reference [74] and reproduced from reference [77].
Figure 1
Interaction of fatty acids with beta cell ultrastructure and function: A: Effects of exposure to 0.33mmol/l oleate or B: 0.33mmol/l palmitate upon the ultrastructure of INS-1 cells. Original photomicrographs provided by Dr Katherine Pinnick and Anne Clark [27]. C: Exposure of mouse islets to oleate or palmitate (0.5mmol/l) for 48h significantly impaired glucose-stimulated insulin secretion (data from K. Pinnick [27]). D: Impairment in glucose-stimulated insulin secretion in mouse islets brought about by incubation for 72h in a fatty acid mixture (0.5mmol/l 1:1/oleate:palmitate) was not present following 48h exposure followed by 24h in fatty acid-free media (reproduced with permission from [27]). E: Effect of exposure of human islets to 0.33mmol/l palmitate on islet triglyceride content and F: islet glucose medicated insulin secretion. Graphs E and F are reproduced with permission from [25].

20x27mm (600 x 600 DPI)
Schematic representation of possible stages of beta-cell fate changes associated with diabetes progression: During metabolic stress, FoxO1 translocates to the nucleus to orchestrate a compensatory response to help preserve glucose oxidation and suppress fatty acid oxidation. FoxO1 target genes include beta-cell transcription factors MafA and NeuroD, which through continued activation of insulin expression help preserve beta-cell function under metabolic stress. Continued stress leads to loss and/or cytoplasmic translocation of beta-cell transcription factors (Nkx6.1 and MafA) and reduced/lost insulin expression. Analysis of human and rodent data indicates that cells, at this stage of diabetes, can undergo significant plasticity events that involves the (re)expression of 'disallowed genes'. At this stage, cells can be characterised by two distinct identities: i) Hormone 'empty' cells that may express progenitor (Ngn3 and Oct4) and mesenchymal (Vimentin) proteins and ii) transdifferentiated/bi-hormonal cells that express other endocrine hormones, including glucagon. Pre-clinical data indicate that recovery of beta-cell failure at this stage of diabetes can be achieved through redifferentiation of dedifferentiated/transdifferentiated cells, a phenomenon that has been implied clinically also following calorie restriction diet. Sustained metabolic stress may result in irreversible beta-cell failure through apoptosis (dedifferentiated cells) and/or established alpha-cell differentiation, however this has yet to be be confirmed in humans. Solid arrows indicate observed rodent and human phenotypes and broken arrows indicate potential cellular pathways that may account for beta-cell loss and/or recovery.

254x190mm (72 x 72 DPI)
During long term excess calorie intake, especially in the presence of muscle insulin resistance, the raised plasma insulin levels will expedite chronic excess calorie storage from carbohydrate via de novo lipogenesis. This will promote storage of fat in the liver very gradually over years and liver insulin resistance, with a consequent tendency for a small increase in plasma glucose (as shown by the Whitehall II study [76]). In turn, insulin secretion will increase to control plasma glucose. The further increased insulin levels will bring about a self-reinforcing vicious cycle. The increased liver fat will inevitably lead to an increased rate of export of VLDL triglyceride from the liver. Along with all other tissue, islets will be therefore exposed to higher rates of fatty acid supply and the exposure of pancreatic endocrine cells to fatty acids and their metabolites will increase. This is postulated to bring about endoreticulum stress in susceptible individuals, and eventually beta cell dedifferentiation, with relative inhibition of meal insulin secretion. The vicious cycles are postulated to interact over many years. At a personal threshold level, it is postulated that the beta-cells can no longer compensate, and plasma glucose levels will then rise relatively rapidly. The Figure is redrawn from reference [74] and reproduced from reference [77].

Figure 3

254x190mm (96 x 96 DPI)