Type 2 Diabetes: The Pathologic Basis of Reversible β-Cell Dysfunction

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Diabetes Care 2016;39:2080–2088 | DOI: 10.2337/dc16-0619

The reversible nature of early type 2 diabetes has been demonstrated in in vivo human studies. Recent in vivo and in vitro studies of β-cell biology have established that the β-cell loses differentiated characteristics, including glucose-mediated insulin secretion, under metabolic stress. Critically, the β-cell dedifferentiation produced by long-term excess nutrient supply is reversible. Weight loss in humans permits restoration of first-phase insulin secretion associated with the return to normal of the elevated intrapancreatic triglyceride content. 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 β-cell response to chronic positive calorie balance.

REVIEW

Until recently, the pathophysiology of type 2 diabetes was believed to be characterized by progressive, irreversible loss of pancreatic insulin secretion (1) mediated by apoptosis of pancreatic β-cells (2). Now that weight loss has been shown to bring about restoration of β-cell function and reversal of diabetes, a fundamental reappraisal of the mechanisms underlying β-cell dysfunction is required. The recent in vivo and in vitro studies will be reviewed, together with the evidence that the underlying, potentially reversible, β-cell failure is related to dedifferentiation rather than to β-cell death.

ETIOLOGICAL DRIVERS OF TYPE 2 DIABETES

Population data demonstrate major increases or decreases in the incidence of type 2 diabetes secondary to food excess or scarcity. This was documented in the U.K. during the First and Second World Wars and in Cuba in 1990–96, with an associated sharp fall in the incidence and prevalence of type 2 diabetes (3,4). Perhaps the clearest evidence for the effect 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 they lived as subsistence farmers (5,6). In 1940, diabetes prevalence was similar to that of the general U.S. population (7). Thereafter, with cessation of an agricultural lifestyle, together with food oversupply, a dramatic increase occurred in the 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 the prevalence in ethnically identical Pima Indians living in Mexico under nutritional conditions that 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 U.K. 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 obesity had no major effect on the development of common type 2 diabetes (9–11). Although this may seem

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Received 21 March 2016 and accepted 23 August 2016.

surprising from a present day perspective, the risk of type 2 diabetes rises most steeply at very high BMIs, and only 7% of the population had a BMI >30 kg/m^2 in 1980 (12). The effect of high BMI on the development of diabetes was simply not detectable. Although this association with obesity is now apparent, it has not generally been recognized that diabetes risk rises steadily throughout the population weight distribution. The Nurses' Health Study showed a fourfold increase in type 2 diabetes prevalence in women with BMI of 23–25 kg/m^2 compared with those with a BMI of <22 kg/m^2 (13). In the UK Prospective Diabetes Study, which recruited between 1977 and 1991, 36% of newly diagnosed individuals had a BMI of <25 kg/m^2 (14). Conversely, 72% of people with a BMI >40 kg/m^2 do not have diabetes (15).

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

THE PATHOLOGIC BASIS OF DISEASE PROGRESSION

At the diagnosis of type 2 diabetes, β-cell function is typically reduced to 50% of normal by HOMA modeling and to a greater extent on dynamic testing (1,18). Despite the initial effect of diet and oral therapy to lower glucose, observational studies have shown that disease progression is associated with inexorably declining β-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 β-cell function and insulin resistance over time. In contrast, the absence of imaging modalities with sufficient resolution to image the β-cell in situ has prevented determination of whether these changes are caused by dysfunction or true loss of β-cell mass at a cellular level (20). Moreover, satisfactory circulating markers of β-cell death or proliferation are currently lacking (21).

Postmortem pancreatic pathology studies, based on presence of staining for cells that contain insulin, have suggested that β-cell mass is significantly reduced in type 2 diabetes in comparison with age-, sex-, and BMI-matched control individuals without diabetes (2). Although it is accepted that increased apoptosis plays a role in decreased β-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 that stain positive for insulin in the pancreatic islets.

GLUCOLIPOTOXICITY

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

Exposure of the rat insulinoma β-cell line to oleic acid brings about storage in intracytoplasmic vacuoles, whereas the saturated fatty acid palmitate induces expansion of the endoplasmic reticulum (Fig. 1*A* and *B*) (27). This is known to be associated with markers of endoplasmic reticulum stress, which are typically elevated in human β-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 the return of insulin secretion over 24 h (Fig. 1*C* and *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 (Fig. 1*E* and *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) that support 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 β-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 VLDL1-triglyceride allows the return of normal insulin secretion in early type 2 diabetes (36,37). A recent study that used optimized pancreatic MRI showed the removable excess of intrapancreatic triglyceride by weight loss was specific to type 2 diabetes (38).

β-CELL DEDIFFERENTIATION

Loss of the fully differentiated phenotype is a recently recognized potential mechanism underlying loss of β-cell function in type 2 diabetes (38–43). The metabolic stress of chronic nutrient oversupply can lead to reduced expression or nuclear activity of key β-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). β-Cell dysfunction ensues from 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 β-cells in vitro after chronic palmitate exposure (46).

Accili and colleagues (47) 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 β-cell transcription factors, including MafA, preserving glucose oxidation, suppressing fatty acid oxidation, and thus limiting

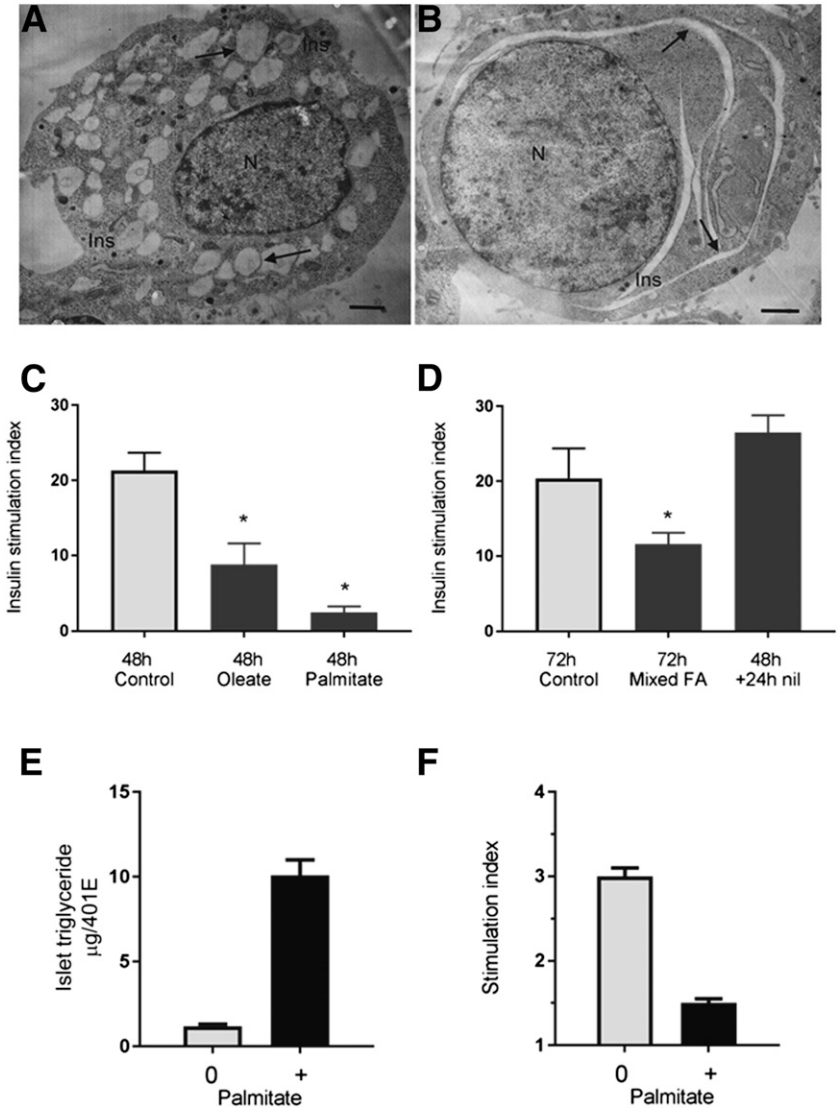

Figure 1—Interaction of fatty acids with β-cell ultrastructure and function. *A*: Effects of exposure to 0.33 mmol/L oleate upon the ultrastructure of rat insulinoma cells. Triglycerides accumulate in round-shaped droplets in the cytoplasm (arrows). *B*: Effects of exposure to 0.33 mmol/L palmitate upon the ultrastructure of rat insulinoma cells. Triglycerides formed splits in the cytoplasm adjacent to the endoplasamic reticulum (arrows). Ins, insulin granules; N, nucleus. Scale bars = 1 μm. Original photomicrographs provided by Drs. Katherine Pinnick and Anne Clark (27). *C*: Exposure of mouse islets to oleate or palmitate (0.5 mmol/L) for 48 h significantly impaired glucose-stimulated insulin secretion (data from Pinnick et al. [27]). *D*: Impairment in glucose-stimulated insulin secretion in mouse islets brought about by incubation for 72 h in a fatty acid (FA) mixture of oleate and palmitate (1:1; 0.5 mmol/L) was not present after 48-h exposure, followed by 24 h in fatty acid–free media (nil) (reproduced with permission from Pinnick et al. [27]). *$P < 0.05$. Effect of exposure of human islets to 0.33 mmol/L palmitate on islet triglyceride content (*E*) and islet glucose-medicated insulin secretion (*F*). Graphs *E* and *F* are reproduced with permission from Lalloyer et al. (25).

mitochondrial stress. Through this mechanism, FoxO1 is able to orchestrate a compensatory response aimed at preserving β-cell function under metabolic stress (Fig. 2). Establishment of chronic hyperglycemia, however, leads to FoxO1 degradation and β-cell decompensation with impaired capacity for glucose oxidation and increased ability to oxidize 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 β-cell–specific deletion of FoxO1 exposed to metabolic stressors, including aging and multiple pregnancies, demonstrated that loss of β-cell mass was caused by dedifferentiation rather than by death (41). While maintaining expression of the endocrine marker chromogranin A, expression of insulin was lost in parallel with a number of key β-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 β-cells, including mesenchymal markers (vimentin) and pancreatic progenitor markers such as neurogenin 3 (Fig. 2). Relevance to type 2 diabetes was evidenced by the demonstration that progressive loss of FoxO1 was associated with a large number of chromogranin A^+/$insulin^-$ 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 β-cells cannot be genetically labeled for in situ lineage-tracing studies in humans, rodent models (39,41,48) have enabled identification of specific transitional phenotypes that can be stained for in pathological pancreatic samples. These can help determine the characteristics and fate of human β-cells during the course of clinical diabetes.

Supported by detailed quantitative human pathology analysis, it is now established that impaired β-cell function in type 2 diabetes cannot be accounted for by increased apoptosis alone (22,49). Recent postmortem studies demonstrate that β-cell dysfunction in type 2 diabetes may be associated with degranulation and alterations in the β-cell phenotype. Expression of key β-cell transcription factors, including PDX1 and MafA, is markedly reduced in type 2 diabetes, suggesting that dedifferentiation (defined by loss of canonical β-cell markers) may contribute to impaired β-cell function (50,51). Furthermore, expression of disallowed genes, including vimentin, has been detected in β-cells from patients with type 2 diabetes, providing support for the observations in rodents made by Talchai et al. (41). In a recent pancreas pathology study, "empty" β-cells were assessed by coimmunofluorescent staining for the endocrine secretory granule marker, synaptophysin, and all endocrine hormones (insulin, glucagon, somatostatin, and pancreatic polypeptide) (52), as described in *db/db* and GIRKO mice. Through use of this approach and normalization to β-cell number, the study determined that the number of "empty" or dedifferentiated endocrine cells was

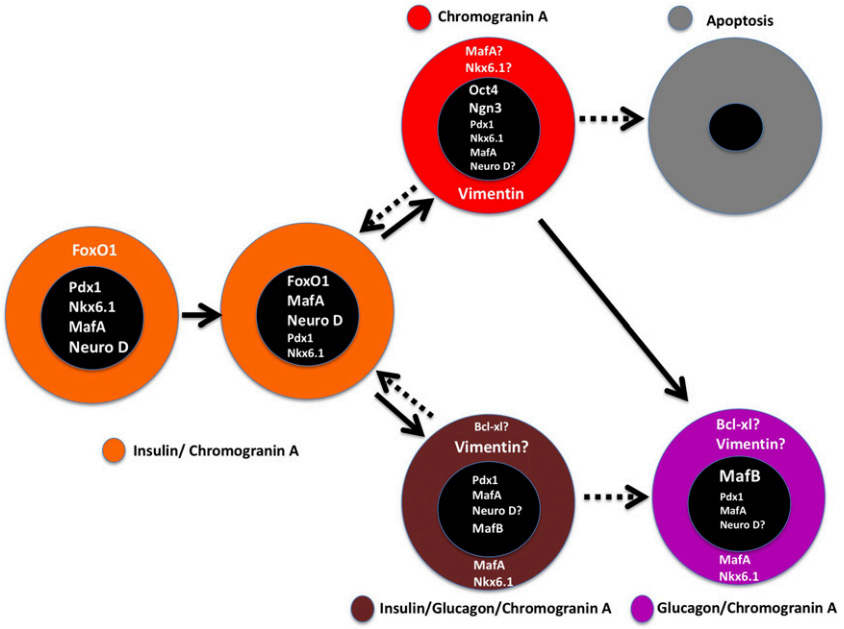

Figure 2—Schematic representation of possible stages of β-cell fate changes associated with diabetes progression. During metabolic stress, FoxO1 translocates to the nucleus to orchestrate a compensatory response to help preserve glucose oxidation and suppress fatty acid oxidation. FoxO1 target genes include β-cell transcription factors MafA and NeuroD, which through continued activation of insulin expression help preserve β-cell function under metabolic stress. Continued stress leads to loss and/or cytoplasmic translocation of β-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 characterized by two distinct identities: *1*) hormone "empty" cells that may express progenitor (Ngn3 and Oct4) and mesenchymal (vimentin) proteins and *2*) transdifferentiated/bihormonal cells that express other endocrine hormones, including glucagon. Preclinical data indicate that recovery of β-cell failure at this stage of diabetes can be achieved through redifferentiation of dedifferentiated/transdifferentiated cells, a phenomenon that has been implied clinically also after a calorie-restricted diet. Sustained metabolic stress may result in irreversible β-cell failure through apoptosis (dedifferentiated cells) and/or established α-cell differentiation; however, this has not yet been confirmed in humans. The solid arrows indicate observed rodent and human phenotypes, and the broken arrows indicate potential cellular pathways that may account for β-cell loss and/or recovery.

significantly increased in people with type 2 diabetes compared with control subjects without diabetes (31.0% vs. 8.7%). Using a similar approach, Butler et al. (53) determined that although altered β-cell phenotypes were evident, this did not account for the β-cell deficit in type 2 diabetes. However, expression of β-cell transcription factors was not assessed within their study, thus limiting complete determination of β-cell (de)differentiation status. In contrast, Cinti et al. (52) were able to demonstrate that a number of β-cells displayed altered subcellular localization of Nkx6.1 and MafA, with expression almost exclusively within the cytoplasm. These findings are consistent with work by Spijker et al. (40) and indicate a functional impairment (caused by cellular localization) of these key transcription factors in human diabetes. The extent to which such alterations contribute to β-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 β-cell dedifferentiation as a mechanism underlying dysfunction have, to date, been unable to elucidate the specific underlying signaling pathways. For example, it is not possible to determine separately the effect of hyperglycemia and dyslipidemia on β-cell (de)differentiation status in *db/db* mice or in pathological samples from human donors with diabetes. Recent studies have sought to explore the effects of individual stresses. For example, nutrient-stimulated insulin secretion was prevented in a transgenic mouse model with an activating K_{ATP} channel mutation of β-cells (39). Hyperglycemia in this model was associated with β-cell dedifferentiation, characterized by the loss of the end-differentiated β-cell markers Pdx1 and MafA. In an ex vivo human islet lipotoxicity model, incubation with palmitate led to β-cell dysfunction associated with loss of the end-differentiated phenotype evidenced by reduced levels of key β-cell transcription factors (46).

HYPERGLUCAGONEMIA IN TYPE 2 DIABETES

Type 2 diabetes has long been recognized to be a dual hormonal disorder, with stimulation of hepatic glucose production by aberrant postprandial hyperglucagonemia playing an important role in overall hyperglycemia (54,55). Whether this state of relative glucagon oversecretion is driven by increased α-cell mass or simply increased function remains unknown. Henquin et al. (56) observed no increases in α-cell mass in patients with type 2 diabetes, concluding that relative hyperglucagonemia was likely being driven by reduced β-cell mass and subsequent alterations in the inhibitory actions β-cells exert on α-cells. In contrast, Yoon et al. (57) observed that reduced β-cell mass was associated with expansion of the α-cell mass in a different cohort of patients with type 2 diabetes. Although it was proposed that increased α-cell mass may be a driver of hyperglucagonemia, a role for reduced β-cell paracrine signaling cannot be excluded in this study.

Given the limitations such cross-sectional human studies pose, definitive evidence for changes in α-cell mass have largely been provided by rodent studies, with recent evidence proposing a potential role for β-cell–to–α-cell conversion as a mechanism underlying this phenomenon (39). Through use of the Fox01 ablation model, Talchai et al. (41) were able to demonstrate that, after metabolic stress, an increase in plasma and pancreatic glucagon was associated with β-cell–to–α-cell conversion, as determined by glucagon expression in β-derived cells. In support of this, and a role for hyperglycemia in these plasticity events, Brereton et al. (39) describe β-cell–to–α-cell

conversion in the K_{ATP} transgenic mouse model. Although describing similar fate-switching events, this study observed a number of bihormonal cells (insulin/glucagon coexpressing), indicating a direct conversion. In contrast, Talchai et al. (41) observed that the expression of glucagon in β-derived cells only occurred once insulin was lost, with no coexpression 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 β-cell stress. What remains consistent, however, is that both studies provide evidence for a shift in endocrine phenotype after the loss of key β-cell transcription factors.

β-Cell dedifferentiation, characterized by the loss of β-cell–specific markers, including Pdx1, Nkx6.1, and MafA, may enable conversion toward other pancreatic endocrine phenotypes that are less susceptible to nutrient-induced mitochondrial and endoplasmic reticulum stress (58). This raises the possibility that β-cell identity may be fragile and that metabolic stress eliminates factors that normally repress non–β-cell identities. In support of this, deletion of Pdx1 in adult rodents leads to severe hyperglycemia in tandem with ultrastructural and physiological α-cell characteristics in a large fraction of the β-cells (48). These data are in line with β-cell–to–α-cell fate-switching events after loss of β-cell–specific transcription factors subsequent to chronic hyperglycemia (39). Collectively, these studies indicate that α-cell and potentially other endocrine cell phenotypes may be "default" lineages and that loss of critical β-cell factors that suppress non–β-cell–related genes, including glucagon, during type 2 diabetes pathogenesis may lead to a shift in endocrine phenotype from β-cell to α-cell (39,41,48). In light of recent data from Marroqui et al. (58) demonstrating that α-cells are more resistant to metabolic stress, it is possible that the transition from β-cell to α-cell during diabetes may be a defense mechanism to maintain cellular mass (58). Because reversal of type 2 diabetes is associated with a fall to normal of fasting plasma glucagon levels at the same time as the return of normal β-cell function, the in vivo human studies may be reflecting redifferentiation of β-cells. No change in plasma glucagon is seen in people with normal glucose tolerance during similar weight loss (59).

In support of earlier work by White et al. (43), a recent study observed an eightfold increased frequency of insulin$^+$ cells coexpressing glucagon in tissue obtained from patients with type 2 diabetes, indicating such β-cell plasticity occurs in human diabetes (40). The study determined that half of the bihormonal (insulin$^+$/glucagon$^+$) cells were negative for Nkx6.1. Furthermore, cytoplasmic localization 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 coexpressed glucagon (52), indicating a role for Nkx6.1 in repressing the α-cell program, as described in vitro (60). 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 bihormonal cells reflect β-cell neogenesis even though this appears unlikely. β-Cell neogenesis has been described as originating close to and within ducts (2,61), and associated bihormonal cells were localized to single and small clusters of β-cells rather than within established islets (61). Human studies using isotopic incorporation into DNA or lipofuscin accumulation indicate no significant β-cell neogenesis occurs in adults (62,63). Also, Nkx6.1 is mislocalized (cytoplasm) in glucagon$^+$ cells (52). Although these postmortem studies cannot provide definitive evidence, a contributory role for β-cell conversion to α-cell in hyperglucagonemia in human diabetes is supported (Fig. 2).

LESSONS ON β-CELL PLASTICITY FROM REVERSING TYPE 2 DIABETES

The Counterpoint study has provided the clearest data on the time course of recovery of β-cell function after calorie restriction in type 2 diabetes. In this study, observations were made at 1, 4, and 8 weeks after commencing a very low-calorie diet (36). After 1 week, there was no improvement in the first-phase insulin response to a stepped insulin secretion test with arginine. This is especially significant because fasting plasma glucose had already normalized 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 the 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 β-cell dysfunction should be rapidly reversible. For instance, the apparent mitochondrial dysfunction of type 2 diabetes is seen only when fasting plasma glucose is $>$8 mmol/L (64) and is rapidly corrected by suppression of plasma fatty acid levels (65). Reversal of oxidative stress and endoplasmic reticulum stress is also associated with rapid restoration of normal β-cell function (27,66).

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 normalization of the first-phase insulin response over 8 weeks was mirrored by a slow normalization of total intrapancreatic fat (36). During this extended interval, the intrapancreatic triglyceride concentration gradually declined to the same level as in control subjects without diabetes (36). The parallel time courses of the fall in excess triglyceride within the pancreas and the recovery of β-cell function are suggestive but not conclusive of cause and effect.

Whether the fall in intrapancreatic triglyceride content could merely reflect the very considerable weight loss and not be related to restoration of β-cell function had to be considered. A further clinical study was conducted to determine whether weight loss also brought about a fall in intrapancreatic triglyceride content in normoglycemic individuals or whether it was specific to type 2 diabetes. The intrapancreatic triglyceride content in a group about to undergo weight loss by gastric bypass surgery was quantified before and 8 weeks after the operation (38). A weight loss of ~13% occurred in those with normal glucose tolerance as well as in those with type 2 diabetes. The former showed no change in intrapancreatic triglyceride, whereas those with type 2 diabetes had higher

levels at baseline, which fell to normal. At the same time, the 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 β-cells to excess saturated fatty acid causes avid uptake and decreases the 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 the liver and the 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 hypothesized to reveal reaccumulation of intrapancreatic fat with or without a decline in β-cell function. This has been examined by 6 months of follow-up after acute weight loss of people with type 2 diabetes (37). The group that achieved a fasting plasma glucose of <7 mmol/L after weight loss demonstrated intrapancreatic triglyceride content falling to normal levels. Critically, weight remained stable over 6 months. The first-phase insulin response became and remained normal in this group. There was no reaccumulation of fat in the pancreas or liver even though the mean BMI was 30 kg/m^2 (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 nondiabetic fasting plasma glucose levels immediately after acute weight loss, only 50% of a long-duration group (8–23 years) did so (67). In an audit of outcomes after bariatric surgery, HbA_{1c} of <43 mmol/mol (6.1%) was achieved by 62% and 26%, respectively, in those with duration of diabetes of <4 or >8 years, respectively (61), reflecting previous observations (68). The Scandinavian Obesity Study also confirmed the importance of duration of diabetes in rates of reversal of diabetes at 2 years (69). 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 after dedifferentiation) and/or irreversible apoptosis (Fig. 2).

Redifferentiation provides an attractive potential underlying mechanism for recovery of β-cell function after a reduction in islet fat content over the time course observed in vivo in humans. It can be hypothesized that this time course is too slow for reversal of acute stress-induced dysfunction and too fast for β-cell neogenesis. The dedifferentiated state could be considered as providing a "hideaway" until the metabolic insult subsides, offering an opportunity for restoration of the end-differentiated state after alleviation of metabolic stresses. This "therapeutic window" appears limited given the failure to rescue β-cell function in subjects with long standing diabetes (59). 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, the stage of diabetes, and the potential for reversal through β-cell redifferentiation (Fig. 2).

The potential for redifferentiation as a mechanism underlying restoration of β-cell function in type 2 diabetes is illustrated by the restoration of β-cell function by intensive insulin therapy in a rodent model of hyperglycemia (42). This restoration in β-cell function was associated with β-cell redifferentiation characterized by increased levels of mature β-cell markers, including insulin, Pdx1, and MafA. These observations support the proposition that after removal of hyperglycemia and change in lipid metabolism achieved by intensive insulin therapy, β-cell redifferentiation 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 after intensive insulin therapy.

It is striking that strategies targeting restored pancreatic insulin secretion directly have not been shown to be disease modifying by restoring functional β-cell mass, although the A Diabetes Outcome Progression Trial (ADOPT) study demonstrated greater preservation of β-cell function with the insulin sensitizer rosiglitazone compared with sulfonylurea secretagogue therapy. Use of exenatide over 3 years caused considerable weight loss and brought about some return of β-cell function (70). Overall, decreased β-cell exposure to nutrient excess appears to facilitate reversal of acute dysfunction and adaptive dedifferentiation (44). Specific therapies targeted toward reversal of β-cell dedifferentiation are needed (71–73). The major question of how function might be returned after apparent end-stage dedifferentiation needs to be addressed.

Identification is underway of candidate pathways that could be targeted to reverse β-cell dedifferentiation associated dysfunction (74). A small molecule inhibitor of the transforming growth factor-β receptor (Alk5) was shown to be capable of reversing β-cell dedifferentiation in islets isolated from mice with extreme diabetes, with urocortin 3 used as a marker for mature β-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 β-cell markers, including insulin, Pdx1, and MafA. However, administration of ALK5 inhibitor to diabetic animals failed to improve glycemic control and caused an overall deterioration in health. This highlights the requirement for development of pathway-specific therapeutics to restore mature, functional β-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 (75). The hypothesis postulated that a chronic positive calorie balance, in the presence of preexisting peripheral insulin resistance and, hence, hyperinsulinemia, would bring about steady accretion of intrahepatic triglyceride and initiate linked vicious cycles (Fig. 3). Although the mechanisms underlying the liver cycle are well established, the recent in vitro work on β-cell dedifferentiation provides an attractive potential explanation for the operation of the

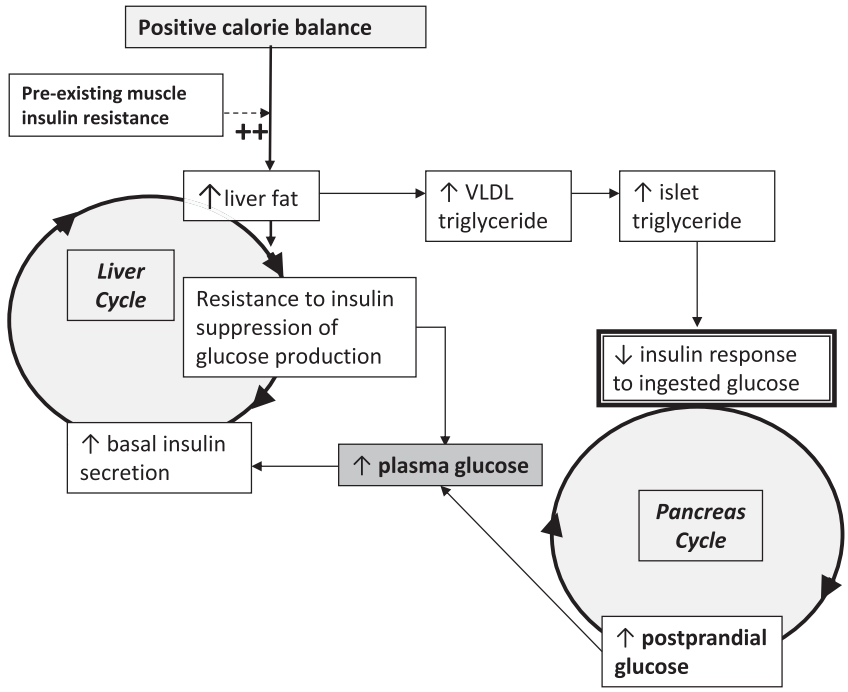

Figure 3—During long-term excess calorie intake, especially in the presence of muscle insulin resistance, the raised plasma insulin levels will expedite chronic excess calorie storage from carbohydrate via de novo lipogenesis. This will promote storage of fat in the liver very gradually over years and liver insulin resistance, with a consequent tendency for a small increase in plasma glucose, as shown by the Whitehall II study (78). 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 therefore be 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 β-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 β-cells can no longer compensate, and plasma glucose levels will then rise relatively rapidly. The figure is redrawn from Taylor (75) and reproduced with permission from Taylor (79).

pancreas cycle (75). The increased plasma VLDL-triglyceride of type 2 diabetes is postulated to bring about fat accumulation and β-cell stress, such that plasma glucose remains elevated for longer after meals. The consequent effects of oversupply of saturated fatty acids on β-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 β-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. In the UK Prospective Diabetes Study population, 36% had a BMI of <25 kg/m^2 at a time when 64% of the whole U.K. population had a normal BMI (12). The proportion of people with type 2 diabetes who are of normal BMI has decreased as the prevalence of overweight/obesity has increased, caused by the exponential relationship between BMI and the prevalence of type 2 diabetes. Between 2000 and 2008, 11.3% those newly diagnosed with type 2 diabetes had a BMI of <25 kg/m^2 compared with 35% in the entire U.K. population (76). Direct observational data suggest that a threshold effect operates—the personal fat threshold concept—whereby ectopic fat accumulation only occurs when an individual's capacity to store fat safely in the subcutaneous compartment is exceeded (37,77). 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 a 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 by β-cell dedifferentiation. The process of losing the specialized 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 β-cell–specific transcription factors and recovery of differentiated glucose-responsive insulin secretion (Fig. 2). The data discussed in this review allow a simplification of understanding of type 2 diabetes, and this will allow reassessment of the many associated processes known to be abnormal.

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

Acknowledgments. The authors are grateful to Drs. Anne Clark and Katherine Pinnick of Oxford University, Oxford, U.K., for permission to publish the original electron micrographs in Fig. 1*A* and *B*, and to Prof. Bart Staels, of The Institute Pasteur de Lille, Lille, France, for permission to reproduce the data shown as Fig. 1*E* and *F*.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.G.W., J.A.M.S., and R.T. each researched published and personal data, jointly synthesized ideas, and wrote the manuscript.

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