Pair Feeding, but Not Insulin, Phloridzin, or Rosiglitazone Treatment, Curtails Markers of β-Cell Dedifferentiation in *db/db* Mice

Emi Ishida, Ja Young Kim-Muller, and Domenico Accili

Diabetes 2017;66:2092–2101 | https://doi.org/10.2337/db16-1213

β-Cell failure is a hallmark of type 2 diabetes. Among several cellular biological mechanisms of cellular dysfunction, we and others have recently proposed that dedifferentiation of β-cells can explain the slowly progressive onset and partial reversibility of β-cell failure. Accordingly, we provided evidence of such processes in humans and experimental animal models of insulin-resistant diabetes. In this study, we asked whether β-cell dedifferentiation can be prevented with diet or pharmacological treatment of diabetes. *db/db* mice, a widely used model of insulin-resistant diabetes and obesity, were either pair fed or treated with the Sglt inhibitor phloridzin, the insulin-sensitizer rosiglitazone, or insulin. All treatments were equally efficacious in reducing plasma glucose levels. Pair feeding and phloridzin also resulted in significant weight loss. However, pair feeding among the four treatments resulted in a reduction of β-cell dedifferentiation, as assessed by Foxo1 and Aldh1a3 immunohistochemistry. The effect of diet to partly restore β-cell function is consistent with data in human diabetes and provides another potential mechanism by which lifestyle changes act as an effective intervention against diabetes progression.

In type 2 diabetes, insulin resistance usually precedes the inception of hyperglycemia (1,2). In the early stages, pancreatic β-cells compensate for the elevated insulin demand to maintain euglycemia. But, as the disease progresses, hyperglycemia becomes increasingly hard to manage (3), in part because of β-cell failure, which attenuates islet insulin secretion (4).

Various mechanisms of β-cell failure have been proposed, including glucolipotoxicity (5), oxidative stress (6,7), endoplasmic reticulum stress (8,9), apoptosis (10,11), or inflammation (6,12). Recently, we and others described β-cell dedifferentiation as a mechanism of β-cell failure in humans (13) and animal models (14–16). We showed that diabetic β-cells lose their identity as insulin-secreting cells and reactivate endocrine progenitor markers, including Neurogenin3 (Neurog3). Dedifferentiated β-cells also give rise to α-cells or other islet cell types.

If β-cells in the diabetic islet are dedifferentiated, and not dead, the question arises of whether β-cell dedifferentiation is reversible. In rodents, there are precedents showing that β-cell redifferentiation can be achieved using insulin or calorie restriction (15–17). In type 2 diabetes, it is well-known that patients' β-cell function can be preserved by diet (18) or by insulin treatment (19,20). Although in the past insulin secretagogues have been suspected to accelerate β-cell failure, newer medications of this class appear to be more durable in this regard (21–23). But the effects of these treatment modalities on β-cell dedifferentiation are still unclear.

Specifically, the relative roles of normalizing glycemia versus reducing insulin resistance have been debated. Modest hyperglycemia has been known to profoundly affect β-cell performance (24); yet, it is undisputable that reducing the "afterload" of insulin resistance also has beneficial effects on the preservation of β-cell function (2). To answer this question, in the current study we aimed to assess the effect of different diabetes therapies on β-cell dedifferentiation in a mouse model of insulin-resistant, obese diabetes: *db/db*. We treated animals with either food restriction by pair feeding or the sodium/glucose cotransporter (Sglt) inhibitor phloridzin, the insulin sensitizer rosiglitazone, and

Department of Medicine and Naomi Berrie Diabetes Center, Columbia University, New York, New York

Corresponding author: Domenico Accili, da230@cumc.columbia.edu.

Received 5 October 2016 and accepted 2 May 2017.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-1213/-/DC1.

insulin. Thereafter, we analyzed basic metabolic parameters and β-cell dedifferentiation markers.

RESEARCH DESIGN AND METHODS

Animals

B6.BKS(D)-Lepr^{db}/J heterozygous mice (*db*/+) were purchased from The Jackson Laboratory (Sacramento, CA) and bred to homozygosity to obtain wild-type, *db*/+, and *db/db* mice. All mice were fed normal chow except in the experiments with rosiglitazone (see below) and maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.). Eight- to 12-week-old *db/db* mice and littermates were subjected to drug treatment or pair feeding and killed after 1 month unless otherwise indicated. Average daily food intake in ad libitum–fed *db/db* mice was 8.5 g, and body weight averaged 55–65 g during treatment. The Columbia University Institutional Animal Care and Utilization Committee approved all experiments.

Study Design

Before treatment, fasting blood glucose and body weight were measured in all *db/db* mice, which were then randomly assigned to control and treatment groups. Likewise, lean littermates were assigned in a blinded manner. Phloridzin (Sigma-Aldrich, St. Louis, MO) was dissolved in 40% polypropylene glycol in saline and injected subcutaneously once daily at a dose of 0.2 g/kg (25). Rosiglitazone (Sigma-Aldrich) was added to normal chow at a dose of 0.15 g/kg. Mice were fed with either regular or rosiglitazone-supplemented chow. The estimated dose of rosiglitazone was 20 mg/kg/day (26). Sustained-release insulin implants, LinBit (15,27), and control implants (Linshin Canada, Inc., Ontario) were placed subcutaneously under the interscapular skin of *db/db* mice. The manufacturer's recommended dosage was adopted. Estimated insulin release from implants was ~0.7 units/24 h. For pair feeding, all the animals were housed individually and fed by food hopper. Cumulative food intake of individual *db*/+ mice was calculated from the total food hopper's weight every 24 h. The next day, the same amount of chow was provided to the paired *db/db* animal. Ad libitum–fed *db/db* mice and controls had free access to food during the experiment. A subgroup of animals did not respond to pair feeding with lower glycemia and were designated as nonresponder (NR) animals.

Metabolic Analyses

Animals were fasted for 5 h before measurement of blood glucose and plasma insulin unless otherwise indicated. We performed glucose tolerance tests in overnight-fasted mice by intraperitoneal injection of glucose (1.2 g/kg). We measured insulin by ELISA (Mercodia, Winston Salem, NC).

Immunofluorescence

Tissues were fixed with 4% paraformaldehyde and embedded in Tissue-Tek O.C.T. Compound to obtain frozen sections. We applied heart-perfused fixation and antigen retrieval to detect transcription factors (Nacalai USA Inc., San Diego, CA) (14). Anti-insulin (category no. A056401-2; Dako, Carpinteria, CA), anti-glucagon (category no. G2654; Sigma-Aldrich), anti-Neurog3 (category no. 2011; Beta Cell Biology Consortium), anti-Pdx1 (category no. 5679S; Cell Signaling Technology, Danvers, MA), and anti-Aldh1a3 (category no. NBP2-15339; Novus Biologicals, Littleton, CO) were used as primary and Alexa Fluor–conjugated goat antibodies as secondary (Jackson ImmunoResearch Laboratories and Molecular Probes) antibodies. For Foxo1 immunostaining, we used a mixture of rabbit polyclonal antibodies, containing antibody nos. 9462 (Cell Signaling Technology), sc-11350 (Santa Cruz Biotechnology, Dallas, TX), and 3587 (28). For Neurog3 immunostaining, we permeabilized the cell membrane by 0.3% TritonX. We used confocal microscopy and Laser Scanning Microscope Software (Zeiss LSM 510 and 710) to survey colocalization and capture images. We performed the quantification in a blinded fashion using the CytoNuclear FL function of the HALO software as previously described (13). The density of immunostaining in the captured images was measured by ImageJ. We defined as "weakly positive staining" a 70% decrease from the maximum density in each islet and as "strongly positive" any cell above this arbitrary threshold.

RNA Analysis

We used an RNeasy Mini Kit (Qiagen, Valencia, CA) to isolate total RNA from islets and High-Capacity cDNA Reverse Transcription kits (Applied Biosystems, Waltham, MA) to carry out reverse transcription. We performed quantitative real-time PCR with goTaq qPCR Master Mix (Promega, Fitchburg, WI) on a Bio-Rad CFX96 real-time PCR system. The relative gene expression levels were determined by the ΔΔCt method using tubulin as a reference gene. The primer sequences are as follows: α-tubulin, forward 5′-GCC TTT GTG CAC TGG TAT GTG-3′ and reverse 5′-CCA CAG AAT CCA CAC CAA CCT-3′; Mafa, forward 5′-ATC ACT CTG CCC ACC AT-3′ and reverse 5′-CCG CCA ACT TCT CGT ATT TC-3′; and Neurod1, forward 5′-GCC CAG CTT AAT GCC ATC TTT-3′ and reverse 5′-CAA AAG GGC TGC CTT CTG TAA-3′.

Statistical Analyses

Sample sizes were estimated from the expected effect size based on previous experiments (13,14). We present data as the means ± SEM. We used two-tailed Student *t* test for data analysis, unless otherwise indicated in the figure legends, and the customary threshold of $P < 0.05$ to declare statistically significant differences.

RESULTS

Characterization of *db/db* Mouse Islets

db/db mice on a C57BL/6J background develop hyperglycemia and hyperinsulinemia at 2–3 months of age (29). We assessed expression of insulin, Foxo1, and the newly identified marker of human and murine β-cell dedifferentiation, Aldh1a3, by immunohistochemistry of islets from ad libitum–fed, 16- to 20-week-old *db/db* mice. Consistent with

prior data from us (13,14,30) and others (17), the number of β-cells expressing cytoplasmic Foxo1 decreased by 26%, whereas the percentage of insulin-positive/Aldh1a3-positive cells increased from 3 to 76% (Fig. 1*A–D*). We detected Neurog3-positive cells in *db/db* mice (Supplementary Fig. 1*B* and *C*). Pdx1 was expressed at similar levels in β-cells in *db/db* and lean mice (Supplementary Fig. 2*A*), whereas MafA and NeuroD1 RNA expression was significantly decreased (16% and 68%, respectively) (Supplementary Fig. 2*B*). Based on these results, β-cells in 16- to 20-week-old *db/db* mice appear to be in the early stages of dedifferentiation. Next, we measured glucagon-immunoreactive cells and found that the percentage of glucagon-positive cells decreased from 13 to 7% (Fig. 1*E* and *F*) ($P = 0.0001$) when normalized by the increased islet size in *db/db* mice. On the other hand, weakly insulin-positive cells increased from 3 to 17% in *db/db* mice (Fig. 1*F*) ($P < 0.0001$). In addition, the percentage of insulin- and glucagon-negative cells increased from 19 to 25% ($P = 0.001$). As the percentage of somatostatin- and pancreatic polypeptide–positive cells was unchanged (data not shown), the likely cause of the increase in hormone-negative cells is a rise in dedifferentiated cells, as shown previously (14). Despite the fact that the percentage of glucagon-positive cells decreased and hormone-negative cells increased, the α-cell–to–β-cell ratio decreased because of the greater number of insulin-positive cells in the larger *db/db* islets (Fig. 1*F*). As previously described (31), the total number of islet cells increased in *db/db* mice (Fig. 1*G*).

Pair Feeding Reverses β-Cell Dedifferentiation

We analyzed the effect of diet on β-cell dedifferentiation in pair-feeding experiments. Food restriction is known to lower blood glucose and increase insulin and Foxo1 expression in *db/db* islets (32). We randomly assigned animals to the pair-fed and ad libitum groups. Pair feeding resulted in an ∼50% reduction of food intake compared with ad libitum *db/db* mice (Fig. 2*A*). After one month of pair feeding, we observed improved glucose tolerance (Fig. 2*B* and *C*) and increased plasma insulin levels (Fig. 2*D*) in a subgroup of animals (responder [R]). Body weight decreased steadily during pair feeding but did not normalize to *db/+* mouse

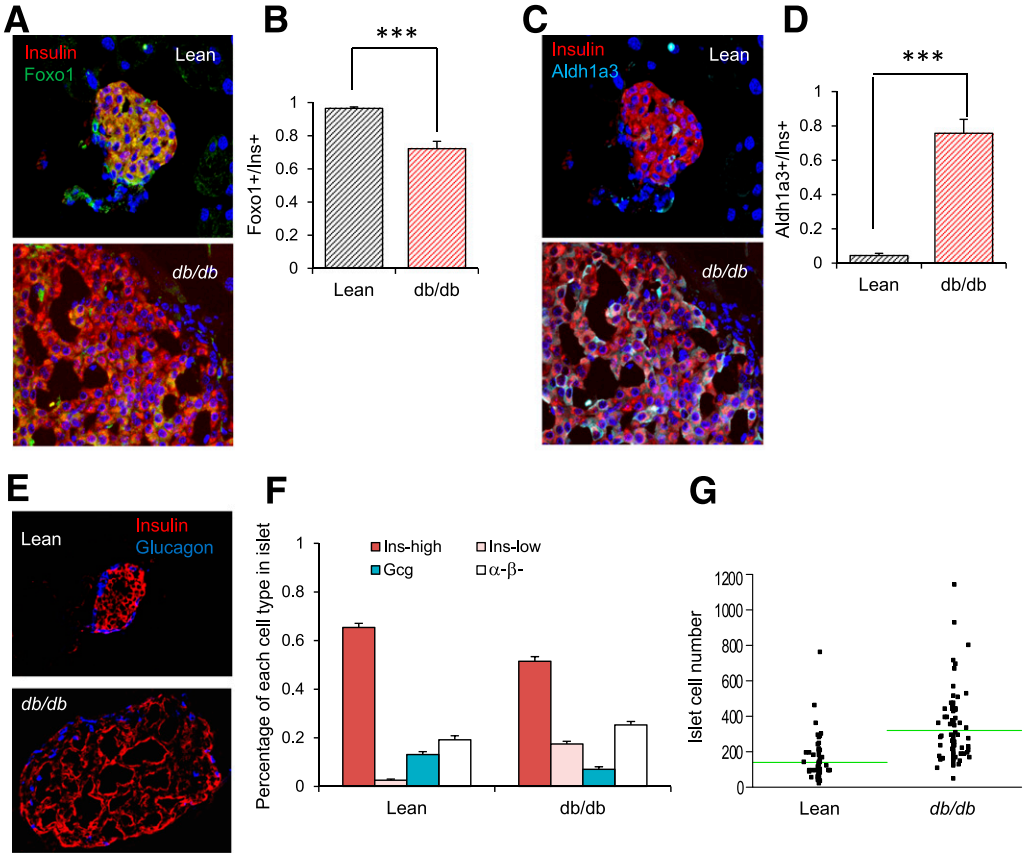

Figure 1—Characterization of *db/db* islets. *A*: Representative images of Foxo1/insulin coimmunostaining. *B*: Number of Foxo1-positive cells among insulin-positive cells. *C*: Representative images of Aldh1a3/insulin coimmunostaining. *D*: Number of Aldh1a3-positive cells among insulin-positive cells. *E*: Representative images of glucagon/insulin coimmunostaining. *F*: Percentage of glucagon-positive and insulin-positive (strong and weak) cells. *G*: Islet cell number in *db/db* animals and controls. Data show means ± SEM. ($n = 11$ for lean group and 14 for *db/db*.) *P* values were calculated by *t* test for *A–F* and Mann-Whitney *U* test for *G* ($P < 0.0001$). ***$P < 0.001$ between the denoted pairs. Gcg, glucagon; Ins, insulin.

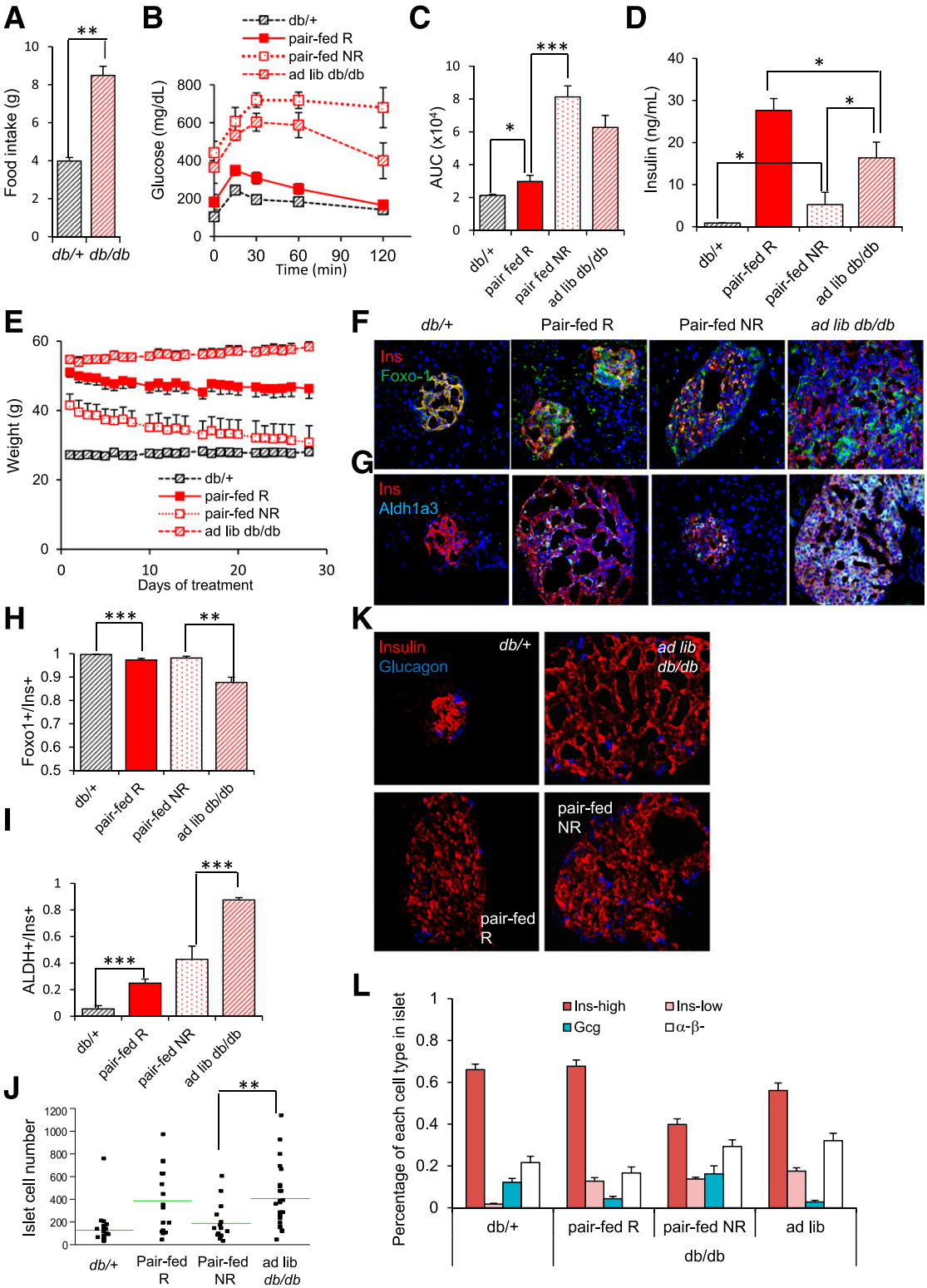

Figure 2—Effect of pair feeding on markers of β-cell dedifferentiation. *A*: Average daily food intake per mouse. *B*: Intraperitoneal glucose tolerance test after 4 weeks of pair feeding. *C*: Area under the curve (AUC) of intraperitoneal glucose tolerance test shown in *B*. *D*: Fasting plasma insulin in the different treatment groups after 4 weeks of pair feeding. *E*: Body weight in the various treatment groups during pair feeding. *F* and *G*: Representative images of Foxo1 (*F*) or Aldh1a3 (*G*) and insulin coimmunostaining in islets of the three groups. *H*: Number of Foxo1-positive/insulin-positive cells. *I*: Number of Aldh1a3-positive/insulin-positive cells. *J*: Total cell number per islet in the various groups after pair feeding. *K*: Representative image of glucagon/insulin coimmunostaining. *L*: Percentage of glucagon-positive and insulin-positive cells. Data show means ± SEM. (n = 6 for db/+, 4 for pair-fed R, 3 for pair-fed NR, and 6 for ad libitum *db/db*.) P values were calculated by t test for *A*–*I* and *L* and Mann-Whitney U test for *J*. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ between the denoted pairs. ad lib, ad libitum; Gcg, glucagon; Ins, insulin.

levels (Fig. 2*E*). A subgroup of animals with hyperglycemia experienced a weight loss that was out of proportion to the pair feeding and appeared to have developed a state of relative insulin deficiency (designated NR).

When we analyzed islets by immunohistochemistry, we found a 17% decrease in Foxo1-positive β-cells that was prevented after pair- feeding regardless of the response on glucose (Fig. 2*F* and *H*). The percentage of Aldh1a3-expressing β-cells decreased from 80 to 25% and 38% in the R and NR groups, respectively (Fig. 2*G* and *I*) ($P < 0.001$). Neurog3-positive cells were absent (Supplementary Fig. 1*D*), and MafA and NeuroD1 expression levels increased in pair-fed *db/db* mice (Supplementary Fig. 2*B*). The number of islet cells, a measure of islet size, was smaller in the pair-fed NR group compared with the ad libitum *db/db* group (Fig. 2*J*). In the pair-fed R group, the percentage of strongly insulin-positive cells per islet increased (56% to 68%, $P = 0.01$) while that of α-cells didn't change (2% to 4%) (Fig. 2*L*). In contrast, the percentage of weakly insulin-positive cells and potentially dedifferentiated insulin- and glucagon-negative cells decreased 18% to 13% and 32% to 17%, respectively ($P = 0.03$ and $P = 0.003$) (Fig. 2*K* and *M*). In the pair-fed NR group, the percentage of non-α/non-β did not change (32% in ad libitum group to 29%), while that of α-cells increased (3% to 16%) ($P < 0.001$).

Phloridzin Does Not Affect β-Cell Dedifferentiation Markers in *db/db* Mice

Phloridzin, a natural flavonoid found in apple bark that inhibits Sglt, thus increasing urinary glucose loss, can normalize glycemia in diabetic rodents, including leptin-deficient mice (33). The relative contribution of hyperglycemia and insulin resistance to the pathogenesis of β-cell dedifferentiation remains unknown. We reasoned that, if hyperglycemia played a dominant role in this process, normalization of plasma glucose by phloridzin should improve β-cell dedifferentiation. Thus, we treated hyperglycemic 8- to 12-week-old *db/db* mice with phloridzin or vehicle for 1 month (Fig. 3*A*). After 30 days of treatment, phloridzin halted the progression of hyperglycemia in *db/db* mice (Fig. 3*B*), without altering plasma insulin levels (Fig. 3*C*), and reduced body weight (Fig. 3*D*). In contrast, vehicle-injected animals developed significant hyperglycemia (Fig. 3*B*). Despite the lower blood glucose, phloridzin did not curtail islet expansion (Fig. 3*E*) or affect Foxo1 (Fig. 3*F* and *G*) or Aldh1a3 (Fig. 3*H* and *I*) expression. Phloridzin slightly increased the percentage of α-cells in *db/db* mice (Fig. 2*J* and *K*), but the change was not statistically significant (6% to 13%, $P = 0.06$); in addition, it had no effect on weakly insulin-positive cells or insulin- or glucagon-negative cells. In sum, phlorizdin surprisingly failed to prevent dedifferentiation markers, despite its profound antihyperglycemic effect.

Effects of Rosiglitazone on β-Cell Dedifferentiation

Given the failure of the glucose-lowering agent phloridzin to prevent β-cell dedifferentiation, we asked whether increasing insulin sensitivity would affect this process. To answer this question, we carried out a 2-month course of rosiglitazone treatment in *db/db* mice and examined metabolic and islet parameters (34). After a 2-month treatment, rosiglitazone improved glucose tolerance in wild-type and *db/db* mice (Fig. 4*A* and *B*), without altering body weight (Fig. 4*C*), and substantially reduced hyperinsulinemia (Fig. 4*D*). The percentage of Foxo1-positive β-cells increased from 56 to 85% (Fig. 4*F* and *G*), but that of Aldh1a3-positive cells did not change, even though it showed a trend toward a modest decrease (Fig. 4*H* and *I*). Rosiglitazone did not change the percentage of glucagon-positive, or weakly insulin-positive, cells or the non-α-cell–to–non-β-cell ratio in *db/db* islets (Fig. 4*J* and *K*).

Effects of Insulin Treatment on β-Cell Dedifferentiation Markers

Finally, we investigated the effect of insulin therapy on β-cell dedifferentiation in *db/db* mice. Although *db/db* mice have severe insulin resistance, insulin administration can be effective in lowering blood glucose levels (35,36). After implantation, blood glucose levels in ad libitum–fed animals fell by ~50% for ~2 weeks (Fig. 5*A*). The number of cells per islet was slightly increased in the insulin-treated group compared with controls but not significantly so (Fig. 5*B*). Foxo1 expression in β-cells was decreased slightly in the insulin group (Fig. 5*C* and *D*). Aldh1a3 expression remained high (Fig. 5*E* and *F*). α-Cell and weakly insulin-positive cell percentage remained the same after insulin implant, as did the non-α-cells/non-β-cells (Fig. 5*G* and *H*).

DISCUSSION

In the current study, we report the effect of different diabetes treatments on β-cell dedifferentiation. In pair-fed *db/db* animals, β-cell markers such as insulin, Foxo1, MafA, and NeuroD1 were restored and the dedifferentiation marker, Aldh1a3, was suppressed. These results are compatible with previous data (17). The percentage of weakly insulin-positive cells decreased, as did the percentage of non-β-cells/non-α-cells, a surrogate of dedifferentiated cells. And there was no increase in α-cells. We also found increased plasma insulin levels, which may reflect improved β-cell function or number, as suggested in a recent study (37). These results suggest that calorie restriction can prevent, and possibly reverse, β-cell dedifferentiation. In a previous study, food restriction in *db/db* mice restored insulin secretion from isolated islets, improved insulin sensitivity, and decreased islet inflammation (32,37). Whether inflammation and dedifferentiation are mechanistically related is unknown. Since Foxo, a driver gene of β-cell fate, is regulated by cytokine signaling (38), the two processes likely overlap.

We have proposed that there are several stages to β-cell dedifferentiation (14). In the first phase, Foxo1 translocates to the nucleus in response to various noxious cues (hyperglycemia, hyperlipidemia, cytokines). Over time, Foxo1 expression decreases as a result of deacetylation, and this is associated with increased expression of the progenitor cell

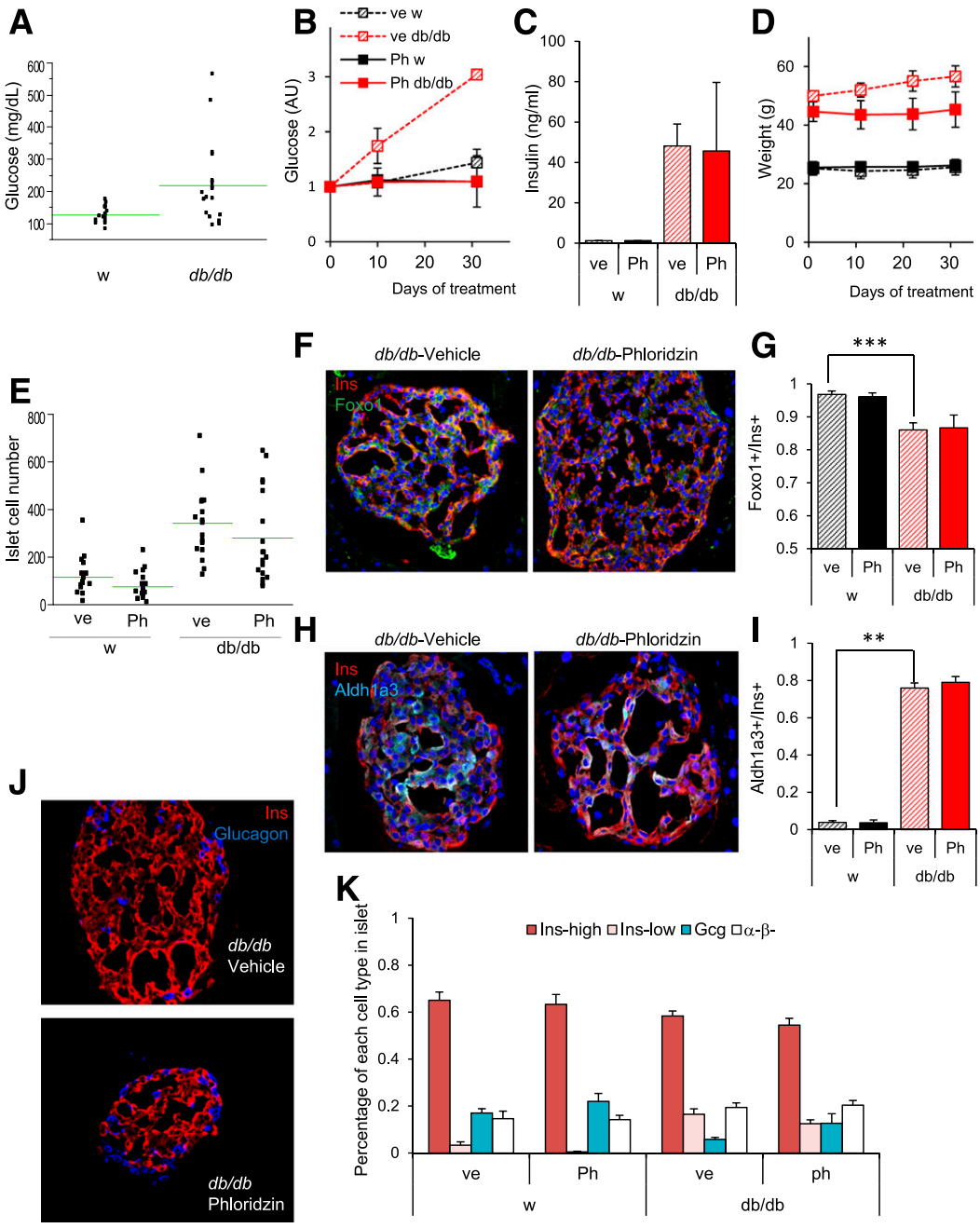

Figure 3—Phloridzin fails to normalize markers of β-cell dedifferentiation. *A*: Blood glucose after a 5-h fast in wild-type (w) and *db/db* mice before treatment ($P = 0.0065$). *B*: Fold change of blood glucose after a 5-h fast during phloridzin (Ph) treatment ($P = 0.0072$ in phloridzin-treated animals vs. controls). *C*: Fasting plasma insulin after phloridzin treatment. *D*: Changes in body weight after phloridzin treatment in *db/db* animals ($P = 0.0032$ in phloridzin vs. vehicle). *E*: Number of cells per islet after phloridzin treatment in both wild-type and *db/db* mice. *F*: Representative image of Foxo1/insulin coimmunostaining. *G*: Number of Foxo1-positive/insulin-positive cells. *H*: Representative image of Aldh1a3/insulin coimmunostaining. *I*: Number of Aldh1a3-positive/insulin-positive cells. *J*: Representative image of glucagon/insulin coimmunostaining. *K*: Proportion of glucagon-positive and insulin-positive cells. Data show means ± SEM. ($n = 3$ for each group.) *P* values were calculated by *t* test for *B*, *C*, and *F–K*; paired *t* test for *D*; and Mann-Whitney *U* test for *A* and *E*. $^{**}P < 0.01$ and $^{***}P < 0.001$ between the denoted pairs. AU, arbitrary units; Gcg, glucagon; Ins, insulin; ve, vehicle.

marker Aldh1a3 and decreased markers of mature β-cells such as Pdx1, NeuroD, and MafA. In the final stages, Neurog3-positive endocrine progenitor-like cells can be found in the islet, as dedifferentiated cells convert to other cell types. By this measure, the islets of mice chosen for the current study are in the early phases of dedifferentiation, as they have low MafA and NeuroD, but are still Pdx1 positive; in addition, Neurog3-positive cells are rare. It should be noted that Aldh1a3 is a marker of a heterogeneous cell population that includes both Pdx1-positive and Neurog3-positive subsets (39).

In clinical practice, evidence that diet improves β-cell function in type 2 diabetes is overwhelming (18). The

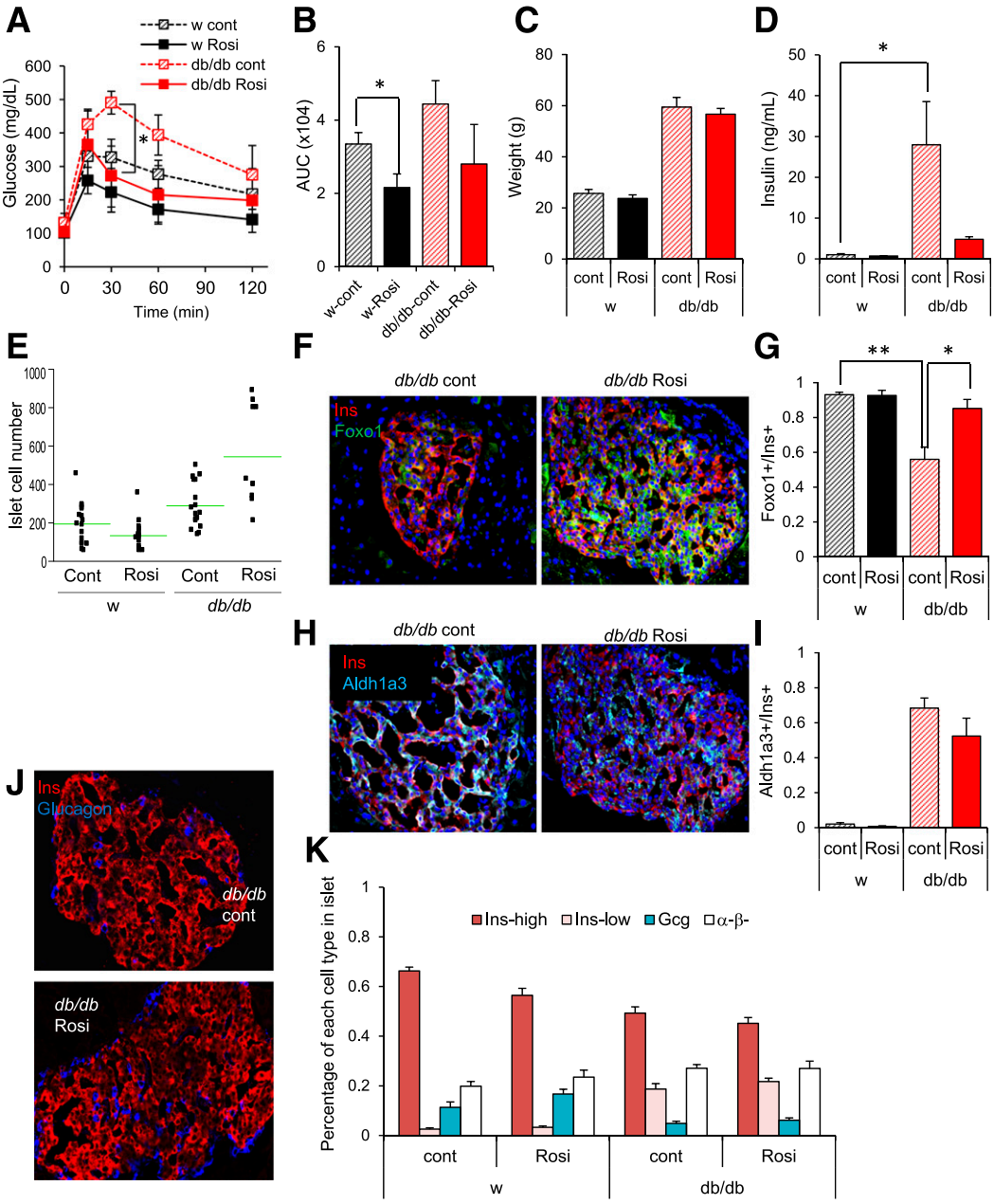

Figure 4—Rosiglitazone fails to normalize markers of β-cell dedifferentiation. *A*: Intraperitoneal glucose tolerance test after rosiglitazone treatment. *B*: Area under the curve (AUC) of intraperitoneal glucose tolerance test shown in panel *A*. *C*: Body weight after rosiglitazone treatment. *D*: Plasma insulin levels after rosiglitazone treatment. *E*: Total islet cell number after rosiglitazone treatment (rosiglitazone vs. vehicle, $P = 0.0159$). Representative image of Foxo1/insulin coimmunostaining (*F*) and cell number (*G*). Representative image (*H*) and number (*I*) of Aldh1a3-positive/insulin-positive cells. Representative image (*J*) and proportion (*K*) of glucagon/insulin-positive cells. Data show means ± SEM. ($n = 3$–4 for each group.) *P* values were calculated by *t* test for *A*–*D* and *F*–*K* and Mann-Whitney *U* test for *E*. $^*P < 0.05$ and $^{**}P < 0.01$ between the denoted pairs. cont, control; Gcg, glucagon; Ins, insulin; Rosi, rosiglitazone; w, wild type.

Diabetes Prevention Trial has consistently demonstrated the superiority of diet and exercise to pharmacological treatment. But we also know that diet alone as a treatment ceases to be efficacious in the more advanced stages of diabetes (40). In humans, reversal of β-cell dysfunction by diet appears to be limited to the first decade of disease (41). Interestingly, severely hyperglycemic *db/db* mice appear to be resistant to pair feeding. The reasons for the failure to respond to pair feeding in a subset of animals may have to do with more advanced disease and functional exhaustion of the endocrine pancreas. In future studies, it will be important to perform a time course to determine whether there is a "point of no return" to restore β-cell dedifferentiation.

Sglt2 inhibitors have made an impact on diabetes treatment, in part due to their ability to forestall cardiovascular mortality (42). In humans, there is evidence that Sglt2 inhibitors protect against β-cell failure (22). In *db/db* mice, phloridzin normalized blood glucose, decreased body

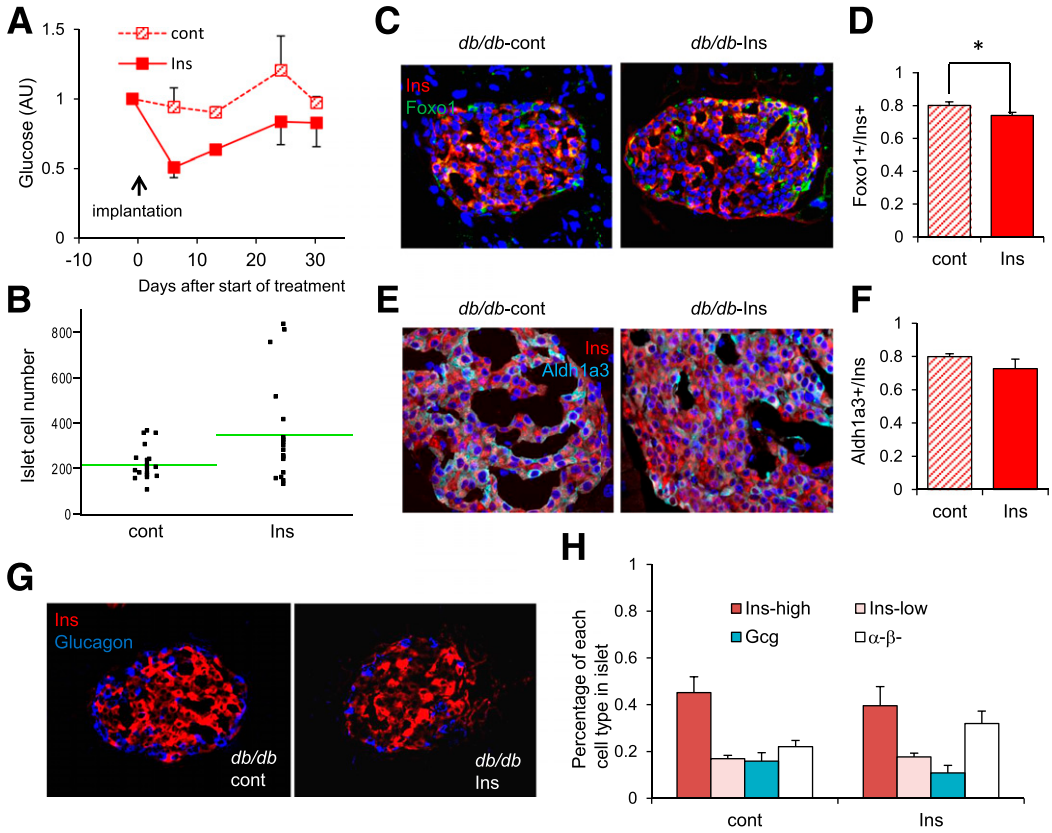

Figure 5—Insulin fails to normalize markers of β-cell dedifferentiation. *A*: Time course of changes in blood glucose during insulin (Ins) treatment in *db/db* mice ($P = 0.0096$ control [cont] vs. insulin). *B*: Islet cell number after 30-day treatment ($P = 0.0875$ control vs. insulin). *C*: Representative image of Foxo1/insulin coimmunostaining. *D*: Number of Foxo1-positive/insulin-positive cells. *E*: Representative image of Aldh1a3/insulin coimmunostaining. *F*: Number of Aldh1a3-positive/insulin-positive cells. Representative image (*G*) and proportion (*H*) of glucagon/insulin-positive cells. Data show means ± SEM. ($n = 2$–3 for each group.) *P* values were calculated by ANOVA for *A*, Mann-Whitney *U* test for *B*, and *t* test for *E*–*H*. $^*P < 0.05$. AU, arbitrary units; Gcg, glucagon.

weight, and improved insulin secretion during glucose tolerance tests without changes to plasma insulin levels or insulin sensitivity (43). But it did not affect β-cell dedifferentiation markers in the current study. In fact, it even tended to increase islet α-cell percentage, possibly as a compensatory response to the lowering of hyperglycemia. These results suggest that alleviating hyperglycemia does not suffice to restore β-cell dedifferentiation. This could be due to the greater effect of pair feeding on lipid levels (32) compared with phloridzin treatment (44), acting possibly to relieve metabolic inflexibility or glucolipotoxicity (30). Alternatively, phloridzin may differ from selective Sglt2 inhibitors used in the clinic. Interestingly, the latter have been found, unlike phloridzin, to increase islet β-cell proportion (43).

Thiazolidinediones (TZDs) primarily act by ameliorating insulin resistance in insulin target tissues. In addition, there is evidence for a direct role of Pparg in β-cells (45), and we have suggested that this is partly due to an effect on lipid disposition (30). Rosiglitazone has been shown to reduce insulin resistance and hyperlipidemia (46) and to improve insulin secretion during glucose tolerance tests in *db/db* mice (47). In the latter study, rosiglitazone also increased islet size and β-cell number. Consistently, we found that it improved glucose tolerance and reduced plasma insulin level, probably as a result of decreased afterload on β-cells. It also restored Foxo1 expression in β-cells but failed to affect other parameters examined. These data are consistent with the findings in insulin-treated animals, in which a reduction of the secretory workload of β-cells was not sufficient to restore β-cell dedifferentiation. It is noteworthy that treating insulin resistance without changes to the diet had a lesser impact on β-cell dedifferentiation than diet alone. The dissociation between increased Foxo1 levels and failure to decrease Aldh1a3 in rosiglitazone-treated *db/db* islet could be interpreted to suggest that rosiglitazone has a partial effect.

TZDs have been reported to improve β-cell function in humans (21,23), but there are no data on dedifferentiation. Thus, it cannot be concluded from these data whether this reflects intrinsic differences among different species or experimental models or a basic mechanism of action. For instance, β-to-α ratio is lower in human islets (48), and humans might respond less to the relative hyperglucagonemia caused by TZDs, compared with mice.

Insulin treatment of *db/db* had the least effect on β-cell dedifferentiation in the current study. *Db/db* mice have

severe insulin resistance and slow-releasing insulin pellets may not have delivered a sufficient amount of insulin to overcome it. In fact, glucose levels did not achieve normal values in the current study. Previous work has shown that insulin supplementation was effective to reverse β-cell dedifferentiation in a model of primary β-cell failure (15). Also, longer (for 12 weeks) and more aggressive insulin treatment successfully restored insulin secretion from isolated islets in *db/db* mice (35). In clinical practice, early intervention with insulin tends to preserve β-cell function and even achieve transient remission (20,21,49).

The other limitation of our study is that we did not use lineage tracing to mark dedifferentiated cells, so we cannot unequivocally assess whether we reversed, as opposed to simply prevented, dedifferentiation. Further work will be required to address this point. Another limitation is that we did not test glucagon-like peptide 1 agonist or dipeptidyl peptidase 4 inhibitors, both of which are known to restore β-cell function and reduce hyperglucagonemia (21,50). A further study is planned to address this question.

In conclusion, we show a remarkable effect of diet on β-cell dedifferentiation in obese mice. Our results strengthen the concept of a central role of nutrition in diabetes management and raise the question of alternative pharmacological approaches to preserving β-cell function and forestalling progression of diabetes.

Acknowledgments. The authors are grateful for members of the laboratory of author D.A. for insightful data discussions. The authors thank Thomas Kolar and Ana Flete-Castro (Columbia University) for outstanding technical support.

Funding. This study was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (DK-64819 and DK-63608) (Columbia University Diabetes Research Center).

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

Author Contributions. E.I. designed and performed experiments, analyzed data, and wrote the manuscript. J.Y.K.-M. designed and generated mice. D.A. designed experiments, oversaw research, and wrote the manuscript. D.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Pajvani UB, Accili D. The new biology of diabetes. Diabetologia 2015;58:2459–2468

2. Ferrannini E. The stunned beta cell: a brief history. Cell Metab 2010;11:349–352

3. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992;340:925–929

4. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802–1812

5. Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr Rev 2008;29:351–366

6. Keane KN, Cruzat VF, Carlessi R, de Bittencourt PI, Newsholme P. Molecular events linking oxidative stress and inflammation to insulin resistance and β-cell dysfunction. Oxid Med Cell Longev 2015;2015:181643

7. Del Guerra S, Lupi R, Marselli L, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 2005;54:727–735

8. Back SH, Kaufman RJ. Endoplasmic reticulum stress and type 2 diabetes. Annu Rev Biochem 2012;81:767–793

9. Fonseca SG, Gromada J, Urano F. Endoplasmic reticulum stress and pancreatic β-cell death. Trends Endocrinol Metab 2011;22:266–274

10. Donath MY, Gross DJ, Cerasi E, Kaiser N. Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes. Diabetes 1999;48:738–744

11. Maedler K, Spinas GA, Lehmann R, et al. Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets. Diabetes 2001;50:1683–1690

12. Donath MY, Størling J, Maedler K, Mandrup-Poulsen T. Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. J Mol Med (Berl) 2003;81:455–470

13. Cinti F, Bouchi R, Kim-Muller JY, et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. J Clin Endocrinol Metab 2016;101:1044–1054

14. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 2012;150:1223–1234

15. Wang Z, York NW, Nichols CG, Remedi MS. Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. Cell Metab 2014;19:872–882

16. Qian B, Zhou X, Li B, et al. Reduction of pancreatic β-cell dedifferentiation after gastric bypass surgery in diabetic rats. J Mol Cell Biol 2014;6:531–534

17. Sheng C, Li F, Lin Z, et al. Reversibility of β-cell-specific transcript factors expression by long-term caloric restriction in db/db mouse. J Diabetes Res 2016;2016:6035046

18. White MG, Shaw JAM, Taylor R. Type 2 diabetes: the pathologic basis of reversible β-cell dysfunction. Diabetes Care 2016;39:2080–2088

19. Glaser B, Leibovich G, Nesher R, Hartling S, Binder C, Cerasi E. Improved beta-cell function after intensive insulin treatment in severe non-insulin-dependent diabetes. Acta Endocrinol (Copenh) 1988;118:365–373

20. Li Y, Xu W, Liao Z, et al. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients is associated with improvement of beta-cell function. Diabetes Care 2004;27:2597–2602

21. Wajchenberg BL. beta-cell failure in diabetes and preservation by clinical treatment. Endocr Rev 2007;28:187–218

22. Merovci A, Mari A, Solis C, et al. Dapagliflozin lowers plasma glucose concentration and improves β-cell function. J Clin Endocrinol Metab 2015;100:1927–1932

23. Hanefeld M, Pfützner A, Forst T, Lübben G. Glycemic control and treatment failure with pioglitazone versus glibenclamide in type 2 diabetes mellitus: a 42-month, open-label, observational, primary care study. Curr Med Res Opin 2006;22:1211–1215

24. Brunzell JD, Robertson RP, Lerner RL, et al. Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. J Clin Endocrinol Metab 1976;42:222–229

25. Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. Diabetes 1999;48:1487–1492

26. Koh EH, Park JY, Park HS, et al. Essential role of mitochondrial function in adiponectin synthesis in adipocytes. Diabetes 2007;56:2973–2981

27. Biddinger SB, Miyazaki M, Boucher J, Ntambi JM, Kahn CR. Leptin suppresses stearoyl-CoA desaturase 1 by mechanisms independent of insulin and sterol regulatory element-binding protein-1c. Diabetes 2006;55:2032–2041

28. Nakae J, Park BC, Accili D. Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. J Biol Chem 1999;274:15982–15985

29. Leiter EH, Coleman DL, Hummel KP. The influence of genetic background on the expression of mutations at the diabetes locus in the mouse. III. Effect of H-2 haplotype and sex. Diabetes 1981;30:1029–1034

30. Kim-Muller JY, Zhao S, Srivastava S, et al. Metabolic inflexibility impairs insulin secretion and results in MODY-like diabetes in triple FoxO-deficient mice. Cell Metab 2014;20:593–602

31. Baetens D, Stefan Y, Ravazzola M, Malaisse-Lagae F, Coleman DL, Orci L. Alteration of islet cell populations in spontaneously diabetic mice. Diabetes 1978;27: 1–7

32. Kanda Y, Hashiramoto M, Shimoda M, et al. Dietary restriction preserves the mass and function of pancreatic β cells via cell kinetic regulation and suppression of oxidative/ER stress in diabetic mice. J Nutr Biochem 2015;26:219–226

33. Khan A, Efendic S. Evidence that increased glucose cycling in islets of diabetic ob/ob mice is a primary feature of the disease. Am J Physiol 1995;269: E623–E626

34. Lohray BB, Bhushan V, Reddy AS, et al. Novel euglycemic and hypolipidemic agents. 4. Pyridyl- and quinolinyl-containing thiazolidinediones. J Med Chem 1999; 42:2569–2581

35. Yates AP, Gordon C, Davies D. Evidence for a direct action of exogenous insulin on the pancreatic islets of diabetic mice: II. Prolonged insulin therapy before islet isolation and perifusion. Diabetes Res 1987;5:89–92

36. Fujita H, Fujishima H, Koshimura J, et al. Effects of antidiabetic treatment with metformin and insulin on serum and adipose tissue adiponectin levels in db/db mice. Endocr J 2005;52:427–433

37. Cheng CW, Villani V, Buono R, et al. Fasting-mimicking diet promotes Ngn3-driven β-cell regeneration to reverse diabetes. Cell 2017;168:775–788.e12

38. Kawamori D, Kaneto H, Nakatani Y, et al. The forkhead transcription factor Foxo1 bridges the JNK pathway and the transcription factor PDX-1 through its intracellular translocation. J Biol Chem 2006;281:1091–1098

39. Kim-Muller JY, Fan J, Kim YJ, et al. Aldehyde dehydrogenase 1a3 defines a subset of failing pancreatic β cells in diabetic mice. Nat Commun 2016;7:12631

40. UK Prospective Diabetes Study Group. UK Prospective Diabetes Study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 1995;44:1249–1258

41. Savage PJ, Bennion LJ, Flock EV, et al. Diet-induced improvement of abnormalities in insulin and glucagon secretion and in insulin receptor binding in diabetes mellitus. J Clin Endocrinol Metab 1979;48:999–1007

42. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2016;374:1094

43. Miyagawa K, Kondo T, Goto R, et al. Effects of combination therapy with vildagliptin and valsartan in a mouse model of type 2 diabetes. Cardiovasc Diabetol 2013;12:160

44. Pei F, Li BY, Zhang Z, et al. Beneficial effects of phlorizin on diabetic nephropathy in diabetic db/db mice. J Diabetes Complications 2014;28:596–603

45. Gupta D, Leahy AA, Monga N, Peshavaria M, Jetton TL, Leahy JL. Peroxisome proliferator-activated receptor γ (PPARγ) and its target genes are downstream effectors of FoxO1 protein in islet β-cells: mechanism of β-cell compensation and failure. J Biol Chem 2013;288:25440–25449

46. Yuan X, Li H, Bai H, et al. The 11β-hydroxysteroid dehydrogenase type 1 inhibitor protects against the insulin resistance and hepatic steatosis in db/db mice. Eur J Pharmacol 2016;788:140–151

47. Ortsäter H, Grankvist N, Wolfram S, Kuehn N, Sjöholm A. Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. Nutr Metab (Lond) 2012;9:11

48. Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M. Islet architecture: a comparative study. Islets 2009;1:129–136

49. Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet 2008;371:1753–1760

50. Hare KJ, Knop FK, Asmar M, et al. Preserved inhibitory potency of GLP-1 on glucagon secretion in type 2 diabetes mellitus. J Clin Endocrinol Metab 2009;94: 4679–4687