Dear Editor,

Type 2 diabetes mellitus (T2DM) develops only in insulin-resistant subjects when pancreatic β-cell compensation fails (Matveyenko and Butler, 2006). Decreased insulin secretory function and reduced cell mass are traditionally viewed as major contributing factors in β-cell insufficiency. A recent study using a diabetic rodent model suggests that progressive β-cell dedifferentiation is an important underlying mechanism in β-cell failure (Talchai et al., 2012). β-cell dedifferentiation in diabetes refers to the loss by healthy β-cells of key components characteristic of the differentiated state (Dor and Glaser, 2013), including insulin (for its secretory product), Glut2 (for glucose intake), and PDX-1 (for critical insulin transcription factor). β-cell dedifferentiation may be largely responsible for not only β-cell secretory dysfunction but also impaired β-cell identity. In view of findings that bariatric surgery in a rodent T2DM model led to increased β-cell mass and improved islet morphology (Strader et al., 2009), we investigated the effects of gastric bypass surgery on dedifferentiated β-cells.

Roux-en-Y gastric bypass (RYGB), a type of bariatric surgery, is an effective surgical treatment for patients with morbid obesity. RYGB surgery also improved secretion of β-cells in response to intravenous glucose (Salinari et al., 2013) and completely resolved T2DM in a significant number of patients (Schauer et al., 2003). In animal studies, novel surgical approaches relieved diabetes in a rapid and sustained manner, independent of weight loss effects (Strader et al., 2009). In view of the trend away from bariatric surgery and toward metabolic surgery, we performed RYGB surgery on spontaneous T2DM Goto-Kakizaki (GK) rats (GK-S), a non-obese model with inherited β-cell deficits, to study weight loss-independent effects of RYGB on islets. Dedifferentiated β-cells were examined in this experimental group (GK-S) and two control groups: (i) sham-operated rats pair-fed with the GK-S group (GK-PF-Sham), and (ii) normal Wistar rats (Wistar).

Pancreatic β-cell function after 3 months of RYGB surgery was evaluated by intravenous glucose tolerance tests (IVGTT). Both blood glucose levels at individual time points and total glucose level determined by area under the curve (AUC) reflected improvement of glycemic controls following RYGB surgery (Figure 1A-i,ii). Basal insulin levels were similar in the two GK groups. Plasma insulin levels after 2 min and 5 min of intravenous glucose load were significantly higher in GK-S group than in GK-PF-Sham group. AUC-insulin level within 5 min was also significantly higher in GK-S group, indicating improvement of first-phase insulin secretion following RYGB surgery (Figure 1A-iii,iv). These findings indicate that pancreatic β-cell secretory function is improved after RYGB surgery, in agreement with previous reports (Salinari et al., 2013).

To distinguish immunoreactive areas between SCG-positive and pancreatic hormone-positive cells, we measured expressions of four pancreatic hormones (glucagon, insulin, somatostatin, pancreatic polypeptide) and SCG in islets by confocal microscopy (Supplementary Figure S1). Cells with strongly positive hormone staining were regarded as healthy endocrine cells, cells with reduced hormone staining were regarded as degranulated endocrine cells, and those SCG-positive but hormone-negative were regarded as dedifferentiated endocrine cells (figure 1C-i; Supplementary Figure S1). Endocrine cells consist of 15–20% α-cells and 65–80% β-cells. Since α-cells determined by glucagon expression display hyperfunction and increased mass in T2DM (Elayat, 1995; Figure 1B-ii and C-iii), we presume that the hormone ‘empty’ and ‘pale-staining’ endocrine cells were primarily dedifferentiated and degranulated β-cells. Islets from GK-S group, in comparison with GK-PF-Sham group, showed a significant reduction in
percentage of degranulated and dedifferentiated immunoreactive areas (Figure 1C-i,ii). Thus, RYGB surgery resulted in a significant increase in the percentage of healthy endocrine cells. We measured individual cell volume and nucleus size of β-cells vs. dedifferentiated β-cells, but did not observe significant differences among three groups (Supplementary Table S1). Total SCGN-
positive endocrine cell mass was significantly increased in GK rats after bypass surgery (Supplementary Table S1). The product of SCGN-positive endocrine cell mass timing percentage of dedifferentiated areas in islets indicated a reduction of total dedifferentiated cell mass after bypass surgery (GK-PF-Sham: 4.13 ± 0.15 mg vs. GK-S: 2.35 ± 0.08 mg; P < 0.05, n = 5). Quantitative PCR analysis of pancreatic tissues, normalized to the geometric mean of the two control genes 18S ribosomal RNA (rRNA) and hypoxanthine-guanine phosphoribosyltransferase (HPRT), showed that gene expression levels of mature β-cell markers (insulin, Pdx-1, Maf A, Pcsk1, Glut2) were significantly increased, while that of the α-cell marker glucagon was significantly decreased in GK rats after RYGB surgery (Figure 1C-iii). Interestingly, SCGN expression levels in three groups were the same, suggesting comparable endocrine cell numbers in equivalent pancreatic tissues from three groups, which was consistent with the data of relative endocrine cell mass (expressed in mg per g pancreas weight; Supplementary Table S1). Levels of mature β-cell markers following RYGB surgery using individual internal standards are shown in Supplementary Figure S1. These findings, taken together, indicate the reduction of pancreatic β-cell dedifferentiation in both frequency and total number following RYGB surgery, consistent with the functional recovery observed after intravenous insulin injection.

Some dedifferentiated β-cells regain markers of their precursors (Xu et al., 2008). We examined the advanced dedifferentiation stages of insulin-depleted β-cells using endocrine progenitor marker Neurog3 (NGN3) and pre-endocrine marker Sox9. If the reduction in ‘empty’ β-cells was resulted from further dedifferentiation to endocrine precursors after RYGB surgery, increased precursors would be observed. Sox9 was barely detected in islets from Wistar, but clearly visible in those from two GK groups (Figure 1D-i). Sox9 clearly plays an important role during pancreas development, but lineage tracing experiments did not indicate that it has any role as a multipotent progenitor in adult β-cells (Kopp et al., 2011). Co-staining for Sox9 and insulin revealed decreased or non-existent insulin staining in Sox9-positive cells (Figure 1D-i), suggesting a compromised β-cell identity stage. To rule out the possibility that Sox9+ duct cells migrate into islets, slides were co-stained with Sox9, committed endocrine marker chromogranin A (Chrg), and 4 hormones. A Chrg+/4-hormones− cocktail was used to define endocrine cells. Sox9+ cells were found to be endocrine-derived, showing Sox9+ in nuclei and Chrg+/4-hormones+ in cytosol (Figure 1D-ii and Supplementary Figure S3A). The significant decreases in total Sox9+ endocrine cell mass and frequency of Sox9+ endocrine cells in islets were consistent with the reduction of β-cell dedifferentiation in multiple stages following RYGB surgery (Figure 1D-ii; Supplementary Figure S3B). PCR data for NGN3, an important downstream signal of Sox9 during fetal pancreas neogenesis, were also consistent with such reduction (Supplementary Figure S3C).

The loss of mature components in β-cells may signal an apoptosis program. Increased apoptosis could cause a reduction of dedifferentiated β-cells. Double staining for insulin and Tunel, which were used to mark apoptotic endocrine cells, revealed that most apoptotic cells showed reduced or vanished insulin staining, indicating that apoptosis is common in unhealthy β-cells (Figure 1E-i). The ratio of apoptotic cells was significantly lower in GK-S islets than in GK-PF-Sham islets (Figure 1E-ii), ruling out the possibility that increased apoptosis contributes to the reduction in degranulated or dedifferentiated β-cells.

In a recent study (Wang et al., 2014), insulin therapy caused redifferentiation of dedifferentiated β-cells. In analogy, we propose that RYGB surgery results in β-cell redifferentiation and subsequent recovery of β-cell identity. To evaluate this possibility, we examined the expression of transcription factor FoxO1, which plays an important role in maintaining β-cell identity and preventing β-cell dedifferentiation under various types of metabolic stress (Talchai et al., 2012). Immunostaining data showed that FoxO1 expression was restricted to nuclei in GK-S islets. FoxO1 showed cytoplasmic localization in Wistar β-cells, but was barely detected in either cytoplasm or nuclei of GK-PF-Sham β-cells (Figure 1F). FoxO1 mRNA expression was also higher following RYGB surgery (Supplementary Figure S4). FoxO1 acetylation, which causes nuclear retention, protects β-cells against oxidative stress by upregulating NeuroD and MafA transcription, and also promotes FoxO1 degradation by targeting Pml bodies (Kitamura et al., 2005). This mechanism is therefore able to protect β-cells against acute metabolic distress but not against the chronic challenges of prolonged hyperglycemia, as indicated by the reduced FoxO1 expression in GK-PF-Sham islets. The finding that FoxO1 expression is maintained in GK-S nuclei is intriguing. The non-downregulated nuclear FoxO1 may continuously reverse deterioration of β-cells that undergo prolonged metabolic stress. Future studies will address the mechanism underlying this effect.

The present findings, taken together, clearly demonstrate a reduction of dedifferentiated pancreatic β-cells in GK rats following RYGB surgery. Coincident reduction of apoptotic β-cells in this group rules out the possibility that increased apoptosis caused the reduction of dedifferentiated β-cells. Rather, this may be resulted from redifferentiation following surgery. These findings help explain the recovery of β-cell function and glucose homeostasis in type 2 diabetic patients following RYGB surgery. Reversing β-cell dedifferentiation state is a promising approach for diabetes therapy.

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