Quo vadis: Where have the \( \beta \)-cells gone?

Islets of Langerhans are a central player for the maintenance of glucose homeostasis in the body. In the case of insulin resistance, large insulin secretion is required by augmenting the functional capacity of single \( \beta \)-cells or increasing the number of insulin-secreting cells. While such compensatory mechanisms for the protection of glucose homeostasis operate in non-diabetic subjects, \( \beta \)-cell inadequacy for insulin resistance represents a key feature in the pathogenesis of type 2 diabetes. Studies in non-diabetic subjects disclosed that the number and mass of \( \beta \)-cells increase in response to an increase in body mass index\(^1\). One might wonder, then, where the new \( \beta \)-cells come from, and how they develop. Efforts to clarify such issues have been hampered, however, as a result of the difficulty of the use of human materials that do not allow longitudinal studies or guarantee of the sample quality for analysis of accurate metabolic and hormonal profiling. By overcoming this difficulty, recent studies by Mezza et al.\(^7\) from Harvard proposed two pathways as a source of new \( \beta \)-cells for compensation: neogenesis from duct cells and transdifferentiation of \( \alpha \)-cells to \( \beta \)-cells (Figure 1).

In the study, the authors took advantage of the opportunity to collect pancreas samples from patients who received pancreatoduodenectomy because of a tumor of the ampulla of Vater. To see the impact of insulin sensitivity on the islet structure, they divided the subjects into two groups of insulin sensitive and insulin resistant subjects. On average, in the 40 days after the operation, the majority (77.7%; 7/9) of insulin-resistant subjects developed overt diabetes, whereas insulin-sensitive subjects remained normoglycemic. To explore the hypothesis that insulin resistance directly contributes to adaptive changes in \( \beta \)-cell mass and function, they measured insulin sensitivity, insulin secretion, and incretin levels before and after the operation. They found that the insulin-resistant group showed a greater reduction in all phases of insulin secretion, and a greater increase in glucagon secretion in response to a mixed meal test after surgery. Concurrently, there was a marked increase in serum glucagon-like peptide-1 (GLP-1), but a decrease in gastric inhibitory peptide (GIP). These changes were associated with larger islets due to \( \beta \)-cell hyperplasia (not due to hypertrophy of \( \beta \)-cells) and increased islet neogenesis. Double staining showed dual reactions to CK19 and insulin, indicating the ducal origin of new \( \beta \)-cells. Markers of \( \beta \)-cell proliferation (Ki67) or apoptosis terminal deoxynucleotidyl transferase dUTP nick endlabeling (TUNEL) were rarely positive. Of note, \( \alpha \)-cells were also increased in the insulin-resistant compared with insulin-sensitive group, and that the \( \alpha \)-cell increase was inversely related with glucose uptake. In addition, glucagon was colocalized with GLP-1 in \( \alpha \)-cells. Interestingly, they found an increased number of double-positive cells for insulin and glucagon in insulin-resistant subjects. They suggested that the relative increase in \( \alpha \)-cell area could lead to an increase in \( \beta \)-cells by transdifferentiation, and also to an increase in intra-islet GLP-1 production.

Thus, the study provided several important messages: (i) insulin resistance directly causes an increase in \( \beta \)-cells; (ii) the new \( \beta \)-cells derive from the ducal wall and transdifferentiation of \( \alpha \)-cells; (iii) they are not supplied by proliferation or replication; and (iv) insulin resistance also increases \( \alpha \)-cells containing GLP-1, which stimulates \( \beta \)-cell neogenesis. As there is no information on the islet structure after the operation, it is not known how the islet endocrine cells undergo the changes after the operation. Nevertheless, their data showing a lack of significant changes in Ki67 and TUNEL staining either in insulin resistant or insulin sensitive groups suggest that neither proliferation nor apoptosis contributes significantly to the \( \beta \)-cell adaptive response to insulin resistance. This concept raised a doubt on the previous findings that \( \beta \)-cell deficits found in type 2 diabetes are mostly attributed to \( \beta \)-cell apoptosis, which was shown in the islet of type 2 diabetic patients by some investigators\(^3,5\). This is not always the case, however, in other studies that could not show apoptotic \( \beta \)-cells in diabetic patients\(^4,5\). Perhaps, TUNEL-positive cells could have been overemphasized in previous studies on humans, though frequently detected in experimental animal models. Hence, \( \beta \)-cell loss should more be critically evaluated to confirm whether the apoptotic cells rarely found in fact contribute to a significant decline of \( \beta \)-cell mass.

Currently, there is a world consensus that there is a significant reduction of \( \beta \)-cell volume density and mass in overt type 2 diabetes\(^4,5\), whereas total islet volume density was not necessarily decreased in previous studies\(^5,8\). Instead, it becomes evident that \( \alpha \)-cell volume density is increased in type 2 diabetes, clinically consistent with hypoinsulinemia and hyperglucagonemia\(^5,6\). The results in non-diabetic insulin-resistant subjects in Mezza’s study\(^2\) are in keeping with the data obtained from type 2 diabetic humans\(^4,5\). Again, the apparent lack of enhanced replication or proliferation of \( \alpha \)-cells in those studies might underscore the role of islet neogenesis and transdifferentiation in the remodeling of type 2 diabetic islets. It is now known that islet endocrine cells regulate the function and proliferation of neighboring cells in a paracrine manner in reciprocal ways\(^7\). Such characteristics of bimodal disorders in type 2 diabetes or insulin resistance might well be accounted for in part by transdifferentiation from \( \beta \)-cells to \( \alpha \)-cells or vice versa (Figure 1)\(^2\). The presence
of endocrine cells double positive for glucagon and insulin could reflect a transitional form of transdifferentiation accompanied by islet neogenesis. An additional important message from their study is the link between α-cells and insulin sensitivity. Their observations on GLP-1 in the islet suggest that α-cells are a potential source of the incretin hormone, which, in turn, can exert a local paracrine effect on the islet function. It is increasingly evident that enhanced glucagon action plays a pivotal role for the onset of diabetes because of the fact that glucagon receptor deficient mice do not develop diabetes after destruction of β-cells by streptozotocin.8 Thus, the exploration of α-cell alterations is critical for a better understanding of diabetes and to develop more effective treatment against hyperglucagonemia. Unfortunately, the current study design is only feasible on cross-sections, and it is not clear how α-cells rich in GLP-1 undergo alterations during development of diabetes.

β-Cell volume density is increased during maturation until the second decade of life, maintained during adulthood and gradually decreased with aging. New β-cells can be formed within the islet, in the ducts and in the exocrine area, showing a small cluster of endocrine cells, which are called neogenetic islets. Although neogenetic islets are reduced with aging, islet neogenesis is commonly encountered in obese persons, patients with gastroenterectomy and pregnant women. In experimental studies on rodents, compensatory β-cell hyperplasia is attributed to increased replication of β-cells, mediated by insulin receptor substrate 2 or glucokinase. Impaired glucose signals in the aforementioned processes could lead to a defective mission for β-cell compensation, resulting in the onset of diabetes. To support this contention, Yoneda et al.10 recently found a high frequency of insulin-positive duct cells, and an increased density of small neogenetic islets in subjects with impaired glucose tolerance and newly diagnosed type 2 diabetic patients, but not any more in long-standing type 2 diabetic patients. The findings are consistent with the results of non-diabetic insulin resistant subjects. It should be of note, however, these two studies that showed insulin-positive duct cells were both carried out on the surgically excised pancreases in which duct systems within the pancreatic parenchyma could be incipiently affected to induce β-cell neogenesis. Further investigations on the pancreas without exocrine pancreatic diseases should be warranted to confirm the presence of islet neogenesis from the duct.

Is the capacity of β-cells for compensation common among different ethnic groups? This question is based on the fact that the β-cell hyperplasia is not robust in obese Japanese, whereas increased β-cell volume density is in parallel with body mass index in American people.13 Although there is no correlation between body mass index and

---

**Figure 1** | Islet remodeling in insulin resistant subjects and type 2 diabetes. Islets become enlarged as a result of increases in β-cells and α-cells in insulin resistant subjects. A recent study by Mezza et al. proposed that new β-cells are derived from ducts and by transdifferentiation of α-cells. Double-positive cells for cytokeratin 19 and insulin, and for insulin and glucagon are encountered more frequently in insulin-resistant subjects compared with insulin-sensitive subjects. In insulin-resistant subjects, α-cells contain both glucagon and glucagon-like peptide-1 (GLP-1). During the development of type 2 diabetes, β-cells once increased will be lost. CK, cytokeratin; IGT, impaired glucose tolerance.
β-cell volume density, comparison between subjects with bodyweight <25 and ≥25 showed a significantly greater β-cell mass in the latter in non-diabetic Japanese, showing a trend that is seen in Americans. Intriguingly, β-cell volume density is commonly reduced in either Japanese with impaired glucose tolerance or Americans with impaired fasting glucose. If this is the case, β-cells in Americans might follow more dynamic alterations compared with those in Japanese. During the process from insulin resistant non-diabetic status to diabetes, β-cells start their journey from birth to the grave changing their phenotype. At the end, will they be lost by cell death, or change into a different cell type? This question could be of paramount importance not only for curiosity, but also for future effective treatment of diabetes. Critical longitudinal analysis will be essential for elucidation of the sequences of β-cell loss in future investigations, by seeking the islet cell composition or the presence of double-positive markers for insulin and glucagon or somatostatin.

ACKNOWLEDGMENT
The authors declare no conflict of interest.

Soroku Yagihashi, Hiroki Mizukami, Wataru Inaba

Department of Pathology and Molecular Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

REFERENCES
1. Saisho Y, Butler AE, Manesso E, et al. β-cell mass and turnover in humans: effects of obesity and aging. Diabetes Care 2013; 36: 111–117.
2. Mezza T, Muscogiuri G, Sorice GP, et al. Insulin resistance alters islet morphology in nondiabetic humans. Diabetes 2014; 63: 994–1007.
3. Butler AE, Janson J, Bonner-Weir S, et al. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52: 102–110.
4. Sakuraba H, Mizukami H, Yagihashi N, et al. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. Diabetologia 2002; 45: 85–96.
5. Mizukami H, Takahashi K, Inaba W, et al. Involvement of oxidative stress-induced DNA damage, endoplasmic reticulum stress, and autophagy deficits in the decline of β-cell mass in Japanese type 2 diabetic patients. Diabetes Care 2014; 37: 1966–1974.
6. Henquin JC, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. Diabetologia 2011; 54: 1720–1725.
7. Rorsman P, Ramracheya R, Rorsman NJG, et al. ATP-regulated potassium channels and voltage-gated calcium channels in pancreatic alpha and beta cells: similar functions but reciprocal effects on secretion. Diabetologia 2014; 57: 1749–1761.
8. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci USA 2010; 107: 16009–16012.
9. Mizukami H, Takahashi K, Inaba W, et al. Age-associated changes of islet endocrine cells and the effects of body mass index in Japanese. J Diabetes Invest 2014; 5: 38–47.
10. Yoneda S, Uno S, Iwahashi H, et al. Predominance of β-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. J Clin Endocrinol Metab 2013; 98: 2053–2061.

Doi: 10.1111/jdi.12287