Currently, one of the most controversial areas in pancreatic islet physiology is the origin of new insulin-producing β-cells that are generated in response to physiological demands postnatally. Harnessing this enigma would provide new means for the treatment of diabetes, a disease characterized by a gradual loss of β-cell mass (type 1 or type 2) and/or a decline in β-cell function combined with insulin resistance (type 2). Restraining destruction and stimulating regeneration of the functional mass would prevail over hyperglycemia and avoid secondary complications such as retinopathy and nephropathy and, potentially, cardiovascular and cerebrovascular diseases (1).

Four potential sources of regenerative β-cells, with their respective advocates in perpetual debate, have been proposed to represent the “Fountain of Youth”: 1) neogenesis of ductal epithelium cells (2, 3); 2) transdifferentiation of exocrine acinar cells (4); 3) differentiation of islet-derived precursor stem cells (5); and 4) β-cell replication. Despite strong evidence for the contribution of neogenesis and transdifferentiation in various models of animal pancreatic injury, recent studies have called into question the role of these entities in β-cell mass expansion in the adult organ (6, 7). Strong skepticism also remains over the potential involvement of progenitor stem cells residing within the pancreas to give rise in vivo to functional β-cells (8). However, β-cell replication, which was disregarded for many years, has lately gained popularity with lineage-tracing studies, demonstrating that preexisting mouse adult pancreatic β-cells were the major source of new insulin-producing cells during adult life (9).

Consistent with animal work, a limited number of human islet studies have confirmed that expansion of the β-cell mass in response to metabolic cues occurs predominantly through cell replication (10–13). A common and important finding of these human as well as animal studies was the correlation between aging and the rapid decline in cell replication. This suggests that the functional β-cell mass is established early in life and thereafter becomes quiescent, with a long turnover and limited β-cell regenerative capacity. Most studies were based on the immunological detection of replicating cells using thymidine analogs such as 5-bromo-2′-deoxyuridine and 5-iodo-2′-deoxyuridine or the proliferation marker Ki67. However, labeling analysis can provide information about new cell formation only at the single cell level. Consequently, it is difficult to gain a view of the proportion of cells that are exchanged over time and to provide an accurate birth date. In fact, very few methods exist to determine the life span of any cell type. One approach employed lipofuscin bodies (LB) as a marker to estimate β-cell longevity. LB accumulate in cells with age. Mathematical modeling of LB accumulation demonstrated that the β-cell mass was established by the age of 20 yr with little adaptive change in response to obesity and diabetes (14). Although informative, these data rely on a reconstructed three-dimensional model of LB and certainly require validation using more direct and robust experimental methods.

In this issue of JCEM, Perl et al. (15) successfully estimated the birth date, turnover, and longevity of β-cells using conventional in vivo thymidine analog labeling initially and then the more audacious technique of retrospective birth dating. In the first approach, the authors profited from historical clinical trials testing the efficacy of thymidine analogs as radiosensitizer in cancer patients to assess the degree of β-cell proliferation and turnover. Immunocytochemistry studies were performed on samples derived from 10 deceased patients who had received thymidine analogs 8 d to 4 yr before death. Only the youngest donors
(ages 18 and 20 yr) exhibited \(\beta\)-cell replication with a significant annual turnover rate of 30 to 60%, whereas older donors (ages 31 to 74 yr) showed no analog incorporation and thus no cell replication. The authors conclude that after age 30, \(\beta\)-cells lose their plasticity under their experimental conditions. To substantiate these findings, Perl et al. (15) used retrospective dating to establish, for the first time, the birth date of \(\beta\)-cells. What exactly is retrospective dating? During the 1950s and early 1960s, the race to nuclear weapons and ultimate world domination by several nations resulted in a dramatic global increase in atmospheric levels of the radioisotope \(^{14}\text{C}\). Subsequent to the signing of the ban treaty in 1963, levels of \(^{14}\text{C}\) were found to exponentially decline with a decrease of 50% approximately every 11 yr due to diffusion as well as equilibration with the environment. Taking advantage of this human mischief, Spalding et al. (16) have shown that the excess \(^{14}\text{C}\) integration into DNA closely correlated with atmospheric levels of the isotope. This relationship was applied to accurately estimate the time point when DNA was synthesized and the birth date of cells (Ref. 16 is a “must read”). Retrospective birth dating is unique because it provides information over the whole life span of a cell population. Perl et al. (15) successfully used this approach to estimate that independent of a donor’s age, \(^{14}\text{C}\) incorporation into \(\beta\)-cell DNA always concurred to levels detected in the first three decades of the individual’s life. Taken together, retrospective birth dating and thymidine incorporation demonstrated that: 1) the average birth date of \(\beta\)-cells is within the first 30 yr of life, with cells having a high rate of turnover; and 2) past the age of 30, \(\beta\)-cell plasticity is lost and remaining cells have a very long life span (15). Although this study may seem only confirmatory of recent publications suggesting that the \(\beta\)-cell proliferative capacity is limited to the first 20 yr of life (or less) (12–14), it is in fact the only one experimentally establishing the age of human \(\beta\)-cells. Nonetheless, these data require confirmation because the study of Perl et al. (15) was limited to 10 donors for analog labeling and only three donors for retrospective birth dating due to technical difficulties in obtaining sufficiently pure DNA for birth dating by \(^{14}\text{C}\).

This bold study raises important questions such as: why do \(\beta\)-cells become quiescent and long-lived when an individual reaches 30? One potential explanation for the gradual loss of the replicative capacity of \(\beta\)-cells may reside in the study by Maedler et al. (11) in which a gradual decrease in the expression of the islet-enriched transcription factor Pdx1 was correlated with the age of donors. Pdx1 was shown to be important not only for \(\beta\)-cell function but also for proliferation and survival (17–19). Thus, a decrease in Pdx1 levels may contribute to a steady decline in the capacity of \(\beta\)-cells to replicate until the age of 30. Thereafter, although sufficient to potentially sustain \(\beta\)-cell functions, Pdx1 levels are no longer high enough to regulate proliferation. It will be of interest to verify this hypothesis and determine whether similar modulations in expression levels of additional transcription factors implicated in \(\beta\)-cell proliferation and survival such as Nkx6.1, FoxM1, and Pax4 are also detected with increasing age (20–22). Another fundamental question that arises is whether a 30-yr-old \(\beta\)-cell can be recruited to proliferate in response to physiological or pathophysiological conditions. As rightfully mentioned by Perl et al. (15), their study unfortunately cannot address this question. The obvious physiological condition is pregnancy, in which insulin output is increased to face demands imposed by the fetus. This increase was shown to be matched by expansion of the \(\beta\)-cell mass in women (23), suggesting that indeed \(\beta\)-cells can be potentially aroused to replicate. A recent study has proposed that serotonin may be implicated in regulating \(\beta\)-cell mass during pregnancy in mice (24). It will be of interest to determine whether serotonin can promote cell replication in human islets isolated from young as well as old donors. An additional argument in favor of the ability of \(\beta\)-cells to reinitiate proliferation past 30 in a pathophysiological setting is the recent study by Willcox et al. (25) showing increased islet cell replication in patients with recent-onset type 1 diabetes. Of particular interest was a female donor 42 yr of age, which exhibited extensive \(\beta\)-cell replication. Interestingly, the authors propose that islet cell proliferation was reinitiated in response to immune-derived signals (25). Consistent with this premise, an independent study recently demonstrated that \(\beta\)-cell replication in nondiabetic donors also correlated with increased inflammatory infiltration associated with prolonged duration on life support (13). It will certainly be of interest to decipher the signals that are involved in reinitiating \(\beta\)-cell replication over age 30.

Thus, to \(\beta\)-e or not to \(\beta\)-e replicating after age 30 remains a debatable question that needs to be further validated experimentally. The study by Perl et al. (15) undoubtedly demonstrates that there is significant \(\beta\)-cell turnover only within the first three decades of life under “normal” physiological conditions. Nonetheless, other studies clearly show that \(\beta\)-cells over 30 can also be recruited to proliferate in response to physiological or pathophysiological situations (13, 23, 25). The challenge is now to decipher the molecular networks regulating \(\beta\)-cell proliferation and survival before and after 30 to develop optimal targeted regenerative therapies for the treatment of diabetes.
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