# β-Cell Turnover

## Its Assessment and Implications

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**The pancreatic β-cells are responsible for the maintenance of the body's glucose levels within a very narrow range; their population is dynamic and undergoes compensatory changes to maintain euglycemia. The structural parameters that allow mass changes (replication, neogenesis, cell volume changes, and cell death) can now be assessed and have proved to be powerful tools. Changes in one parameter can dramatically affect the β-cell mass. Unfortunately, conclusions are often drawn on measurements that do not assess β-cell mass but only relative volumes. Throughout the lifetime of a mammal, low levels of β-cell replication and apoptosis are balanced and result in a slowly increasing mass. The balance allows gradual replacement of the β-cell population; thus, β-cells should be considered a slowly renewed tissue. Two major implications of β-cell turnover are that *1*) at any time, the β-cells would be at different ages and *2*) any limitation on replacement could have dire consequences for glucose homeostasis. *Diabetes* 50 (Suppl. 1):S20–S24, 2001**

The evidence that the β-cell mass exists in a dynamic state is becoming increasingly strong. Accumulated experimental data from rodents show that β-cells change in mass and function to maintain plasma glucose levels in a very narrow range. The structural parameters that allow mass changes (replication, neogenesis, cell volume changes, and cell death) have been recognized for many years, but only in the last decade have integrated analyses of these parameters been done. From these studies, several concepts have emerged:

- There is growth of the β-cell mass throughout the life span.
- There is continued renewal and loss, that is, turnover, of β-cells.
- There exist additional compensatory changes to maintain glucose homeostasis.

This perspective will address the basis of these concepts, address the assessment of the components that regulate β-cell mass and turnover, and discuss the implications of having a slowly renewed and ever-expanding β-cell mass.

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IRS, insulin receptor substrate.

### ASSESSMENT TOOLS FOR β-CELL TURNOVER

To be able to document β-cell turnover, there are a number of parameters one must quantitate: β-cell mass, β-cell number, and β-cell replication. Two other parameters, β-cell neogenesis and β-cell apoptosis, can be documented for frequency, but rates for these cannot as yet be measured.

The assessment of β-cell mass has proved to be a powerful tool. Unfortunately, often conclusions have been drawn on measurements that do not assess β-cell mass but only relative volumes. To estimate β-cell mass, one must have the pancreatic weight as well as quantitate the relative volume (also called relative density, volume density, or percentage of pancreatic tissue) of the β-cells. The pancreatic weight can be equated with pancreatic volume or mass if one makes the reasonable assumption that 1 $cm^3$ tissue weighs 1 g. With data for relative volume and pancreatic weight, one can estimate the absolute weight or mass of the β-cells (Fig. 1). Many different methods for quantitating the relative volume of a tissue exist: point counting, linear scanning, and planimetry; each of these methods determines a percentage or proportion of the tissue that the component comprises. As illustrated in Fig. 2, the same absolute mass/weight of a component will correspond to a different relative volume/density depending on the size or volume of the whole. Therefore, values of relative density, whether given as percent pancreas or islets per millimeter squared, cannot be compared among experimental groups unless one is sure that the pancreas weight does not change. Insulin content per pancreas has been used as a surrogate for β-cell mass quantitation, but because β-cells can become degranulated when exposed to hyperglycemia, this measurement may or may not give a good estimation. Islet size differences certainly reflect changes in β-cell mass, but accurate measurement of them is difficult because of the nonrandom location of the islets and the distribution of islet size.

β-Cell number can be estimated by dividing the β-cell mass by the mean individual cell volume. Cell volume is estimated from cross-sectional cell area and the volumetric equation for the volume of a sphere. The volume of a sphere is a cubic function, so seemingly small differences in cross-sectional area can be significant in volume. Cross-sectional area can be measured at the light microscopic level or, with greater precision, at the ultrastructural level (1–3). Errors inherent in either method will be consistent within an experiment, so data can be compared among groups if obtained with the same technique. Because fixation artifacts, such as shrinkage, can influence these measurements, it is imperative to process controls and experimentals together.

The gold standard to quantitate the number of replicating β-cells has been incorporation of tritiated thymidine or bromodeoxyuridine (BrdU) to label those cells in the S phase (DNA

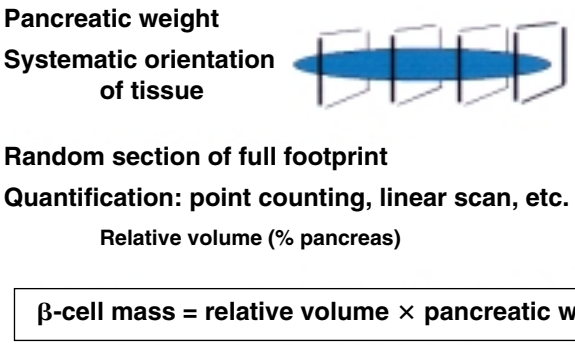


**FIG. 1. Current determination of β-cell mass. To estimate β-cell mass, one must have the pancreatic weight as well as quantitate the relative volume (also called relative density, volume density, or percentage of pancreatic tissue) of the β-cells. The sampling of tissue should be systematic but random. Many different methods for quantitating the relative volume of a tissue exist: point counting, linear scanning, and planimetry; each of these methods determines a percentage or proportion of the tissue that the component comprises. With data for relative volume and pancreatic weight, one can estimate the absolute weight or mass of the β-cells.**

synthesis) during the exposure. Rat islet cells (presumably β-cells) have a cell cycle of 14.9 h (4,5). We have often used a 6-h incorporation time ($G_2 + M = 6$ h) to maximize the number of β-cells labeled without having labeled daughter cells; incorporation of longer times includes variable numbers of labeled daughter cells that confound the comparison to other studies. There are various markers for cell cycle proteins that are useful for retrospective analysis of replication. Proteins expressed at certain times in the cell cycle, such as Ki67 and PCNA, have been useful when indicating cells in the cell cycle. However, because they are expressed from mid-$G_1$ to mitosis, such data cannot be compared with BrdU data.

Neogenesis, or new differentiation of islet cells from ductal epithelium, is seen postnatally as endocrine cells budding from the ducts or scattered as single or doublet cells within the pancreas. Neogenesis has been stimulated experimentally (6–11) and is seen in normal postnatal development (12,13). Quantification of neogenesis has been difficult but has usually been achieved by distinguishing between extra-islet and intra-islet endocrine cells (10,14).

Apoptosis in vitro can be synchronized and studied with many techniques. However, within the native pancreas β-cell, apoptosis is seen at low levels; therefore, morphological techniques are the most useful. The TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling) technique has been used extensively, but the caveat of false positives due to DNA repairing or to DNA fragmentation of necrosis must be remembered (15,16). The more classic morphological methods of identifying highly condensed and/or fragmented nuclei (apoptotic bodies) can be enhanced with DNA dyes such as propidium iodide, Hoechst dye, or DAPI (4′,6-diamidino-2-phenylindole); these DNA dyes are compatible with immunostaining of the islet cells. However, none of these techniques can give a rate of apoptosis, only a frequency, because there are no data on the length of time that apoptosis lasts or that apoptotic nuclei are visible in vivo.

## EVIDENCE OF β-CELL TURNOVER

In the rat, the replication of β-cells drops from a perinatal high of ~18% new cells per day to ~7% at 31 days and 2–3% per day in the adult rat (2,17). This low replication rate has led many to assume that the β-cell mass one is born with is all the β-cells one will ever have. This conclusion ignores that the β-cell mass continues to grow in adulthood. Compilation of data from male Sprague-Dawley rats showed that there was an increase in β-cell mass with age from birth until 6–10 months (13), with a linear increase from about 1 month (weaning) until 6 months; a recent study (18) shows that in Lewis rats, the β-cell mass continues to increase even up to 20 months of age. With even a 2–3% proliferation, the β-cell mass would almost double within 1 month if there were no cell loss (12). If the cell loss equaled the cell renewal, there could be complete replacement in 1 month. A doubling occurs in the young adult rat but does not continue past 4 months of age even though the replication rate remains similar. This lack of continued doubling indicates slow loss of β-cells coincident with the proliferation. Indeed, in the adult rat, apoptotic β-cells had a frequency of 0.5%. The slow continued increase in β-cells until 15–20 months (18) suggests that cell renewal must exceed cell loss until that age. The steady-state balance allows gradual replacement of the β-cell population; thus, β-cells should be considered a slowly renewed tissue.

## DYNAMICS OF β-CELL MASS

Based on data on the normal growth of the rat endocrine pancreas, a mathematical model of the dynamics of β-cell mass was constructed (13). This model facilitates estimations of the changes due to neogenesis and cell death that are difficult to quantitate; the model is expressed as follows:

$$\text{Replication} + (\text{neogenesis} - \text{death}) = \frac{\text{d}(\beta\text{-cell mass})}{\text{d}(\text{time})}$$

This equation emphasizes the different parameters involved in the maintenance of the β-cell mass and how changes in one parameter can dramatically affect it. This model predicted a neonatal remodeling of the endocrine pancreas. Throughout the neonatal period, when there is a high level of replication of β-cells, apoptotic β-cells are threefold (1.5%) more frequent than those in the adult rat (2). Between 13 and 17 days,

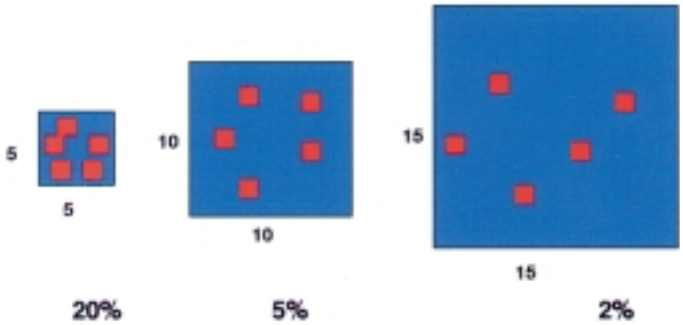


**FIG. 2. Absolute volume can be unchanged as relative volume (volume density/number of millimeters squared) changes. The same absolute mass/weight of a component (the five red boxes) will comprise a different relative volume/density depending on the size or volume of the whole. Relative volume/density is a proportional value, such that in the smallest blue square, the red boxes comprise 20% of the total, but in the largest square, they comprise only 2%. The values of relative density, whether given as percent pancreas or islets per millimeter squared, cannot be compared among experimental groups unless one is sure that the pancreas weight does not change.**

the incidence of apoptosis was even greater (3.6%). This loss coincides with changes in the availability of IGF family survival factors (19), suggesting that the remodeling resulted from an inadequacy of survival factors (2,20). Increased and persistent circulating IGF-II in transgenic mice suppressed the normal neonatal β-cell apoptosis, thus supporting this hypothesis (21). In addition, IGF family survival factors may be important at other times. The survival of differentiating β-cells is impaired in insulin receptor substrate (IRS)-2 null mice, and this seems to be signaled through the IGF-1 receptor (22–24).

In both the rat (18) and the mouse (25), β-cell mass is linearly related to body weight from 1 month of age onward. In addition, the mass of β-cells is dynamically adjusted to maintain euglycemia and therefore must be considered a compensatory factor for glucose homeostasis (26). Glucose infusion for 96 h causes both enhanced β-cell replication and hypertrophy, resulting in a 50% increase in β-cell mass; however, β-cell function is impaired (1). With cessation of infusion, function is restored within 24 h (27), but involution of the β-cells is not seen at 7 days but evidenced by 30 days (1). Similarly, in pregnancy, there is a 50% increase in β-cell mass, with larger islets, increased β-cell replication, and hypertrophy of β-cells (28–30). Additional functional compensation is seen as changes of threshold for glucose-induced insulin secretion (31). During lactation, the β-cell mass returns to nonpregnant levels; this involution results from decreased replication, reduced cell volume from a hypertrophied state to a transient slightly atrophied one, and increased apoptosis (30,32,33). After transplantation of insulinomas, there is involution due to apoptosis of endogenous β-cells (34), and after the removal of insulinomas, there is an expansion of β-cell mass due to changes in cell size and number (35). In all these cases, involution of β-cell mass is far slower than its expansion.

### β-CELL COMPENSATION FOR INSULIN RESISTANCE

Compensatory increases in β-cell mass are found with insulin resistance. Evidence from human autopsied pancreas suggests a compensatory growth of the β-cell mass in obesity (36). Mice with known causes of insulin resistance, due to the ablation of one allele of the insulin receptor, of IRS-1, or both of these genes, are a good case study of the drive to maintain euglycemia in face of insulin resistance (37). Up to 6 months of age, these mice have body weights comparable to their wild-type littermates and remain normoglycemic in spite of marked insulin resistance. Plasma insulin levels are up to 400-fold increased compared with those of the wild-type littermates. β-Cell mass was up to 40-fold increased in mice with double heterozygosity of the null genes. These mice had very large islets and no change in the mass of the non–β endocrine cells, i.e., the α-, δ-, and PP-cells. The massively enlarged islets support a continued stimulation of replication of the preexisting β-cells as the compensatory mechanism.

The Zucker diabetic fatty (ZDF) rat has been another excellent model of compensatory changes of β-cell mass. While all Zucker fatty (*fa/fa*) rats have severe insulin resistance due to a mutated leptin receptor, only male rats from the colony of GMI (Indianapolis, IN) became diabetic; females, even from this colony, remained near-normoglycemic. The rats of this colony must have other genetic mutations because in the prediabetic 6-week-old ZDF male rats, the β-cells have an intrinsic secretory defect compared with their lean ($fa^+/fa^-$ or $fa^+/fa^+$) littermates or the nondiabetic Zucker fatty rats (38). At 6 weeks of age, male Zucker fatty and ZDF rats both have increased body weight, insulin resistance, and a twofold increased β-cell mass compared with the lean littermates of the ZDF rats. At 12 weeks, male ZDF rats are diabetic and have doubled their β-cell mass compared with that at 6 weeks, but the nondiabetic Zucker fatty rats have increased their β-cell mass fourfold. Because the replication and cell volume were comparable but the increase in mass was impaired in the ZDF rats, increased apoptosis was implied but could not be detected, suggesting that either the increase was slight over the whole 6-week period or that a more abrupt loss quickly preceded the onset of diabetes. Further experiments with the female ZDF rats and their lean littermates at 12 weeks of age suggest a greater vulnerability in the highly compensated β-cells (39). The 12-week female ZDF rats had increased body weight and increased β-cell mass compared with the lean littermates and only slightly impaired glucose tolerance. They were thus compensated with hypertrophied β-cells and an increased replication rate compared with the lean animals. After 48 h of treatment with dexamethasone, the lean Zucker females increased β-cell mass because of the compensation by β-cell hypertrophy and increased replication. In contrast, the ZDF females became diabetic. Their already hypertrophied β-cells did not increase in size; their replication was enhanced, but because of the increased frequency of apoptosis, the β-cell mass did not increase. These data suggest that hypertrophy may provide a benefit in terms of increased insulin secretion but may be detrimental in terms of vulnerability to stress, such as glucocorticoids.

### IMPLICATIONS OF β-CELL TURNOVER

The two major implications of β-cell turnover are that *1*) at any time, the β-cells would be at different ages and *2*) any limitation on replacement could have dire consequences for glucose homeostasis. Because the steady-state β-cell replication rate in the rat is just over 2% per day, the life span of a rat β-cell can be estimated as ~58 days (13). Because the replication rate of human β-cells is far lower (40,41), the expected turnover time would be far longer with a greater life span. Whereas it is known that fetal and neonatal islets do not show glucose-induced insulin response, it is unclear if there are other functional differences due to cell age. Because we do not have markers for the age of β-cells, we can only speculate on what differences age could make on the functional status of the cells. Age may be the basis for the secretory and biosynthetic heterogeneity that has been reported for β-cells (42). It may result in differences in susceptibility to damage. Cells can probably respond to stimuli with replication for only a finite number of cycles after which they are senescent or irreversibly unable to replicate. At senescence, the cell may acquire characteristics not found in replicative cells, previously termed "terminally differentiated."

The limitation of the number of possible replications would confer advantage to other means of increasing the β-cell mass. An increase in cell size without replication (hypertrophy) could be an efficient and economical mechanism for rapid and transient compensation. Because hypertrophy is reversible, there may be an economy, since cells with limited replications are not forced to proliferate only to be deleted shortly afterward. Alternatively, as suggested by the reliance on β-cell hypertrophy to increase β-cell mass in very

old rats (18), this mechanism may be very important for maintaining glucose homeostasis once most of the cells are senescent and can no longer replicate. It is important to note that our mathematical model does not account for changes in cell volume as a mechanism to add β-cell mass. Hypertrophy has been recognized in many rat models (1,3,10,18,30,38,39), but its regulation in β-cells is only now being addressed. In muscle and renal tissue, hypertrophy has been seen as a rapid response to increased demand, with an arrest in $G_1$ of the cell cycle (43,44). The mechanisms of hypertrophy involve cell cycle kinase inhibitors (cdks) that interfere with the normal progression of the cell cycle from $G_1$ to S. The combination of initiating $G_1$-associated cell growth (protein synthesis) and preventing DNA synthesis leads to $G_1$ arrest and sustained amplification of gene expression. However, hypertrophy has been seen as an early compensatory mechanism that may eventually progress to loss of functioning tissue (45) because the cells may be more vulnerable to apoptosis (46). In the two clear examples of rapid loss of β-cells—the *db/db* mouse on the C57Bl/Ks background (47) and the desert sand rat (*Psammomys obesus*) (48)—it is unknown if the β-cells are hypertrophied before their rapid decompensation or involution.

Neogenesis may remain a more expandable means of generating new β-cells. In the embryo, neogenesis is the major mechanism for forming new β-cells, and this capacity is maintained after birth. Immediately after birth, many new islets are formed, and a second wave of neogenesis occurs around weaning (30). As seen experimentally, the adult duct epithelium retains the ability to give rise to all the differentiated cell types of the pancreas (49). Neogenesis of islets probably occurs at a low frequency through much of the adult life. In the normal adult pancreas, the ducts are quiescent, being restrained by local factors. If they are isolated from their stroma and put into primary culture, the ducts proliferate extensively. There is a similar rapid expansion of the duct cells during the regeneration after partial pancreatectomy in the adult rat, even at age 6–10 months. As duct cells replicate, they transiently express the transcription factor PDX-1/IDX-1 (50), which is expressed in the embryonic pancreatic ducts but repressed in the ducts shortly before birth. We have hypothesized that with rapid replication, mature duct cells can transiently regain a less differentiated and less restricted phenotype and thus serve as the functional stem cells of the adult pancreas (50). External stimuli, whether soluble factors or matrix components, can then direct differentiation of these multipotent cells to endocrine, acinar, or mature duct phenotypes; in vitro cultivation of human islets from human ductal tissue has been possible (51). Whereas this pathway has considerable capacity for generation of islet tissue, the in vivo environment is carefully regulated to prevent an excess of islet tissue with a potential resulting hypoglycemia.

Diabetes results from an inadequate mass of functional β-cells. Such inadequacy could result by several mechanisms: lack of compensation to overcome the insulin resistance or an intrinsic β-cell defect, as seen in ZDF rats; loss of β-cells, whether from fragility, shortened life span, or immune assault; or lack of survival as new cells differentiate from ductal precursors. The parameters that regulate β-cell mass and turnover surely have genetic basis; it will be intriguing to unravel the genes involved.

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