Linear Correlation Between β-Cell Mass and Body Weight Throughout the Lifespan in Lewis Rats
Role of β-Cell Hyperplasia and Hypertrophy

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We determined the β-cell replicative rate, β-cell apoptosis, cross-sectional β-cell area, and pancreatic β-cell mass throughout the entire postweaning lifespan (months 1, 3, 7, 10, 15, and 20) of Lewis rats. β-Cell replication was progressively reduced in the initial months of life but remained stable after month 7 (month 1, 0.99 ± 0.10%; month 3, 0.24 ± 0.04%; month 7, 0.12 ± 0.02%; month 10, 0.14 ± 0.02%; month 15, 0.10 ± 0.03%; month 20, 0.13 ± 0.03%; analysis of variance [ANOVA], P < 0.001). β-Cell apoptosis was low and did not change significantly from month 1 to 20 of life. Cross-sectional area of individual β-cells increased progressively in the initial months, remained stable from month 7 to 15, and increased again on month 20. The estimated number of β-cells per pancreas, calculated as the ratio of total β-cell mass to individual β-cell mass, tripled from month 1 to 7 but did not change significantly thereafter. β-Cell mass increased ~8 times from month 1 to 20 (month 1, 2.04 ± 0.28 mg; month 20, 15.5 ± 2.32 mg; ANOVA, P < 0.001) and showed a strong and significant linear correlation with body weight (r = 0.98, P < 0.001). In summary, we have shown that β-cell replication was maintained throughout the lifespan in normal rats, clearly establishing that the β-cell birth rate does not fall to 0, even in very old rats. β-Cell mass increased throughout the lifespan, closely matching the increment in total body weight at any time point. This increment was selective for β-cells, since the growth of the endocrine non-β-cell mass was limited to the initial months of life. Both β-cell hypertrophy and hyperplasia contributed to increased β-cell mass in young animals, but only β-cell hypertrophy was responsible for the increased β-cell mass found in old animals. This study provides a global perspective for understanding the dynamics of β-cell mass in young, adult, and aged animals. Diabetes 49:1341-1346, 2000

The β-cell mass present in the pancreas plays an essential role in determining the amount of insulin that is secreted, and it can be regulated to maintain euglycemia in various metabolic conditions (1,2). Increased β-cell mass has been consistently observed when islets must meet an increased metabolic demand in obesity (3,4), pregnancy (5), partial pancreatectomy (6), glucose infusion (7), or islet transplantation (8). Conversely, a reduction in β-cell volume has been reported in chronically hypoglycemic animals bearing transplantable insulinomas (9,10).

Although changes in β-cell mass have been studied for years, important questions still remain about their regulation (11). β-Cell mass is determined by the size and number of β-cells, and the number of β-cells depends on the balance among β-cell neogenesis, β-cell replication, and β-cell death. It is generally accepted that in the fetal period, most β-cells are formed by differentiation from precursor cells and that after birth, most new β-cells result from replication of pre-existing β-cells (12). Therefore, in the postnatal period, the balance between β-cell replication and death largely determines the number of β-cells. In rodents, β-cell replication is high during late gestation and in the neonatal period, is reduced after weaning, and it is usually believed that there are not major changes after 30-40 days of age (11,13). However, the evolution of β-cell replication throughout life has not been specifically studied, and there is a surprising absence of data on β-cell replication in adult and old animals. Recently, a mathematical model of the dynamics of β-cell mass in the normal rat pancreas was constructed, assuming that the β-cell replication rate falls exponentially to a constant level as a function of age (11). However, it was specifically pointed out that because of the lack of data beyond 100 days of age, it could not be ruled out that the replicative rate fell to 0, whether this occurred is a key question in understanding the dynamics of β-cell mass. In addition, limitations in β-cell replication could contribute, as suggested, to the development of diabetes in adult or old subjects only if replication is maintained throughout life (14,15). Thus, there is a clear need for a prospective study on the evolution of β-cell replication covering the unexplored adult and old periods of life.

β-Cell apoptosis plays an active role in determining the dynamics of β-cell mass in normal rat pancreas in the neonatal (16) and postpartum (17) periods, during which increased apoptosis has been shown to determine the remodeling of β-cell mass. However, β-cell apoptosis has not been deter-
mined in old animals, and it is not known whether it actively contributes to changes in β-cell mass. Changes in the balance between β-cell replication and apoptosis would increase or reduce β-cell mass and could theoretically contribute to the development of diabetes. Thus, the aim of the study was to determine the β-cell replicative rate throughout the entire postweaning life in a single rat strain, and, in particular, to establish whether β-cell replicative capacity was maintained even in late ages of life. In addition, we sought to determine whether β-cell apoptosis played an active role in the dynamics of β-cell mass after the neonatal period. Finally, we determined individual β-cell size to obtain an integrated view of the contribution of β-cell replication, β-cell size, and β-cell apoptosis to the changes of β-cell mass throughout life.

**Research Design and Methods**

Male Lewis rats (B & K Universal, U.K.) were studied at 1, 3, 7, 10, 15, and 20 months of age. The β-cell replication data from 2 groups of 4- and 11-month-old Lewis rats previously reported by our group (18) were also included. Animals were kept under conventional conditions in climatized rooms with free access to water and standard pelleted food. Body weight and food intake were recorded. Blood glucose were determined every month between 9:00 and 11:00 a.m. Blood was obtained from the snipped tail with a heparinized microcapillary tube, and glucose was measured with a portable glucose meter (Accu-Check II, Boehringer Mannheim, Mannheim, Germany).

**Pancreas Harvesting.** Six hours before pancreas excision, rats were injected with 5-bromo-2′-deoxyuridine (BrdU) (100 mg/kg body wt i.p.; Sigma Immunochemicals). Under anesthesia with sodium thiopental (100 mg/kg i.p.) (Pentothal Sódico; Abbott Laboratories, Madrid, Spain), a midilaparotomy was performed to expose the pancreas. The animals were killed, and the pancreas was immediately dissected from surrounding tissues, cleared of fat and lymph nodes, blotted, weighed, and fixed in Bouin’s solution. Pancreases of animals older than 1 month were divided into 2 blocks, and the weight of each block was determined on a Mettler balance type A240 reading to 0.01 mg (Mettler Instruments, N.J.). Fixed pancreases were washed with water and stored in 10% buffered formalin until processed for paraffin embedding. The histology procedures were uniform throughout the study.

**β-Cell Replication.** To determine β-cell replication, 3-µm sections of pancreas were double stained with immunoperoxidase for BrdU (a thymidine analog incorporated into newly synthesized DNA of cells in the S phase of the cell cycle) and for the endocrine non-β-cells of the islets, as previously described (18). Immunostaining for BrdU was performed with a Cell Proliferation Kit (Amersham International, Amersham, U.K.); sections were incubated with a mouse monoclonal antibody anti-BrdU for 60 min at room temperature, washed with phosphate-buffered saline (PBS) (pH 7.4), incubated with an affinity-purified peroxidase anti-mouse Ig, and stained with 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma Immunochemicals) plus a substrate intensifier containing hydrogen peroxide and nickel chloride/cobalt chloride. The sections were then stained for the endocrine non-β-cells of the islets with a cocktail of antibodies (Dako, Carpinteria, CA): rabbit anti-porcine glucagon (final dilution 1:1,000), rabbit anti-human somatostatin (final dilution 1:1,000), and rabbit anti-human pancreatic polypeptide (final dilution 1:500). The sections were incubated overnight at 4°C with this cocktail, washed with PBS, and incubated 1 h at room temperature with a swine anti-rabbit IgG used as a secondary antibody. The sections were stained with DAB and hydrogen peroxide (Merck, Darmstadt, Germany). β-Cells and BrdU-β-cells were counted using a Olympus BH-2 microscope connected to a video camera equipped with a rhodamine filter. The results were expressed as the percentage of apoptotic over total cell nuclei.

**β-Cell Number per Pancreas.** β-Cell number per pancreas was calculated as the ratio of total β-cell mass to individual β-cell mass. Individual β-cell mass was calculated as the product of individual β-cell volume to β-cell density, assuming that density was similar for endocrine and exocrine tissue and did not change with age in adult animals (20). The density of 1.0 mg/ml was used for calculations. The individual β-cell volume was derived from the cross-sectional area of individual β-cells, assuming that β-cells are spherical (21). The following equations were used to calculate β-cell number.

**RESULTS**

**Blood glucose, body weight, and pancreas weight.** All animals remained normoglycemic throughout the follow-up (Table 1). Two animals, aged 16 and 18 months, respectively, died spontaneously, and death was considered related to aging. Body weight increased progressively, with a maximum value on month 20 that was significantly higher than that on month 15 (ANOVA, P < 0.001; Fisher’s PLSD, P < 0.05 between month 20 and 15) (Table 1). In contrast, pancreas weight increased markedly from month 1 to month 3 but did not show any further increase thereafter (ANOVA, P < 0.001, between month 1 and all other months).

**Islet morphology.** Islet morphology in the first year of age was that commonly reported for rat islets, with a core of insulin-stained β-cells and a mantle of non-β-cells. In older rats, islets were larger and more irregular in shape, with streaks of fibrotic tissue disrupting the core of β-cells.

**β-Cell Mass and Non-β-Cell Mass.** β-Cell mass increased ~8 times from month 1 to month 20 (Fig. 1) (month 1, 2.04 ± 0.28 mg; month 3, 5.92 ± 0.88 mg; month 7, 10.2 ± 1.04 mg; month 10, 11.0 ± 0.34 mg; month 15, 13.6 ± 3.21 mg; month 20, 15.5 ± 2.32 mg [ANOVA, P < 0.001]). β-Cell mass showed a strong and significant linear correlation with body weight, both when individual animals were considered (r = 0.83, P < 0.001) and when age-groups were analyzed (r = 0.98, P < 0.001) (Fig. 2). The β-cell mass-to-body weight ratio
remained constant throughout life (month 1, 22 ± 3 × 10⁻⁶; month 3, 21 ± 2 × 10⁻⁶; month 7, 22 ± 2 × 10⁻⁶; month 10, 22 ± 1 × 10⁻⁶; month 15, 25 ± 4 × 10⁻⁶; month 20, 22 ± 3 × 10⁻⁶), indicating overall that β-cell mass shows an exquisite adaptation to changes in total body weight.

In contrast, the endocrine non–β-cell mass increased from month 1 to 7 (month 1, 0.66 ± 0.13 mg; month 3, 1.06 ± 0.26 mg; month 7, 2.54 ± 0.30 mg) but remained stable thereafter (month 10, 1.92 ± 0.30 mg; month 15, 2.50 ± 0.18 mg; month 20, 2.04 ± 0.25 mg) (ANOVA, P < 0.001; Fisher’s PLSD, P < 0.05 between months 1 and 3 and all other months). The percentage of non–β-cells in the islet decreased from month 1 to 3 (24.3 ± 2.0 vs. 14.4 ± 2.5%, ANOVA, P < 0.03) and remained stable thereafter. When the endocrine non–β-cell mass was expressed per gram of body weight, it showed a tendency toward a progressive reduction (month 1, 6.5 ± 1 × 10⁻⁶; month 3, 3.8 ± 1 × 10⁻⁶; month 7, 5.6 ± 1 × 10⁻⁶; month 10, 3.7 ± 0.5 × 10⁻⁶; month 15, 4.8 ± 1 × 10⁻⁶; month 20, 2.9 ± 0.5 × 10⁻⁶; ANOVA, P < 0.05).

**β-Cell replication.** β-Cell replication in the 2 previously reported groups (4-month-old, 0.28 ± 0.06%; 11-month-old, 0.16 ± 0.03%) was similar to that of month 3 (0.21 ± 0.04%) and month 10 (0.12 ± 0.01%) groups, respectively, and for these pairs (3- to 4-month-old and 10- to 11-month-old), the results were pooled. β-Cell replication was progressively reduced in the first months of life but remained stable after month 7 (month 1, 0.99 ± 0.10%; months 3 and 4, 0.24 ± 0.04% month 7, 0.12 ± 0.02% months 10 and 11, 0.14 ± 0.02% month 15, 0.10 ± 0.03% month 20, 0.13 ± 0.03%; ANOVA, P < 0.001; Fisher’s PLSD, P < 0.05 between months 1 and 3 and all other time points) (Fig. 3).

**Apoptosis.** The percentage of apoptotic β-cells did not change significantly from month 1 to month 20 of life (month 1, 0.11 ± 0.02% month 3, 0.05 ± 0.02% month 7, 0.20 ± 0.07% month 10, 0.10 ± 0.02% month 15, 0.13 ± 0.04% month 20, 0.20 ± 0.07%).

**β-Cell size and number of β-cells.** Cross-sectional area of individual β-cells increased in the initial months of life and then remained stable from month 7 to 15 (month 1, 100 ± 6 µm²; month 3, 116 ± 2 µm²; month 7, 143 ± 5 µm²; month 10, 132 ± 6 µm²; month 15, 157 ± 14; ANOVA, P < 0.001; Fisher’s PLSD, P < 0.05 between months 1 and 3 and all other groups). The β-cell size increased again on month 20 (186 ± 8 µm²; Fisher’s PLSD, P < 0.05 vs. all other months) (Fig. 4). The number of β-cells per pancreas tripled from month 1 to 7 (month 1, 2.78 ± 0.48 × 10⁶ cells; month 3, 6.20 ± 0.87 × 10⁶ cells; month 7, 7.98 ± 0.95 × 10⁶ cells; ANOVA, P = 0.0035; Fisher’s PLSD, P < 0.05 between month 1 and all other groups), but did not change significantly thereafter (month 10, 9.53 ± 0.69 × 10⁶; month 15, 8.42 ± 1.25 × 10⁶ cells; month 20,

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood glucose (mmol/l)</th>
<th>Pancreas weight* (mg)</th>
<th>Body weight* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5.4 ± 0.1</td>
<td>543 ± 32†</td>
<td>101 ± 2†</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4.6 ± 0.3</td>
<td>1,317 ± 78</td>
<td>277 ± 12†</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>4.1 ± 0.1</td>
<td>1,123 ± 34</td>
<td>455 ± 12</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>4.3 ± 0.2</td>
<td>1,277 ± 63</td>
<td>512 ± 24</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>4.8 ± 0.2</td>
<td>1,146 ± 90</td>
<td>522 ± 65</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>5.0 ± 0.3</td>
<td>1,293 ± 66</td>
<td>713 ± 28†</td>
</tr>
</tbody>
</table>

Data are n or means ± SE. *ANOVA: P < 0.001; †P < 0.05 compared with all other groups.
8.38 ± 1.74 × 10^6 cells). Thus, both β-cell hypertrophy and hyperplasia contributed to increased β-cell mass in young animals, but only β-cell hypertrophy was responsible for the increased β-cell mass found in old animals.

DISCUSSION

The mass of pancreatic β-cells is dynamic and can be modified to maintain normoglycemia in response to changes in metabolic demand. β-Cell mass depends on the changes in β-cell formation (replication and differentiation), individual β-cell size (hyperplasia or atrophy), and β-cell death. The balance between these elements determines whether β-cell mass is increased, remains stable, or is reduced. In this study, we have determined β-cell replication, individual β-cell size, β-cell death, and β-cell mass from month 1 to 20 of life in a single rat strain. In addition, we have estimated the number of β-cells present in the pancreas in each time point.

This is the first study to show that β-cell replication is maintained throughout the entire lifespan in normal rats and that β-cell replication remains stable after young adulthood, clearly establishing that β-cell birth rate does not fall to 0, even in late periods of life. Swenne (13) showed that β-cell replication is reduced with aging, but data were restricted to the fetal period and young adulthood, and the evolution of β-cell replication in older animals is unknown. If β-cell replication were maintained in normal adulthood and aging, limitations in β-cell growth could play a role, as suggested, in the development of type 2 diabetes (14,15). The possible contribution of defects in β-cell replication to the development of diabetes has been based on the reduced β-cell mass found in type 2 diabetic patients compared with weight-matched non-diabetic subjects (4,22,23). In addition, insufficient β-cell replication has been reported in several experimental models of type 2 diabetes, such as male OLETF rats (24) and streptozocin-treated neonatal rats (25,26).

Previous studies showed that the calculated β-cell birth rate decreased from 15.5% in fetal islets to ~3% per 24 h in normal young adult rats (13). Our incorporation of BrdU after a 6-h exposure would correspond to a replication rate of ~4% per 24 h in 1-month-old rats, ~1% in 3- to 4-month-old rats, and ~0.5% in adult and aged rats. Thus, our results in 1-month-old and 3- to 4-month-old Lewis rats are similar to those reported in rodents of similar age (11). In contrast, β-cell replication in adult and aged Lewis rats was <3% which has often been considered the normal β-cell replication rate in adult rodents. However, this replicative rate is not based on direct measurement but on the assumption that there is little change in β-cell replication rate beyond 30–40 days of age (11). In agreement with our results, Swenne and Andersson (27) found a decline in β-cell replication between 3 and 9 months of age in C57BL/6 mice—a strain with normal β-cell replicative capacity. Moreover, Lewis rats are not known to have any limitation in β-cell replication; on the contrary, we have shown that Lewis rats have a substantial capacity to increase β-cell replication in response to increments in metabolic demand (8,19).

Therefore, we believe that our results reflect the normal evolution of β-cell replication in rodents and indicate that β-cell replication in adult and aged rodents is significant, although lower than generally considered. β-Cell replication in human islets was reported to decrease with age and to be lower than that in rodents (28). However, the cell birth rate of human β-cells was 0.4% a value similar to the replication rate found in this study in adult and aged rodent islets, suggesting that β-cell replication of human and rodent β-cells may be closer than previously thought.

β-Cell apoptosis is low and similar in young, adult, and more interestingly, old rats. Available data on β-cell apoptosis in normal pancreas are restricted to the initial months of life (3,16,17,29,30). Scaglia et al. reported increased apoptosis in neonatal rats around days 13–20 of life (16) and in the physiological involution of β-cell mass that occurs postpartum (17). In all other physiological conditions, apoptosis was low or even undetectable in normal pancreas (3,16,17,29,30), as confirmed by our results. The stable values of β-cell apoptosis in young, adult, and aged rats suggest that apoptosis did not play an active role in determining the modifications of β-cell mass after weaning. However, because apoptosis is generally accepted to be a very rapid process, we must caution that relatively small changes in the apoptotic rate might not be detectable. On the other hand, although β-cell neogenesis cannot be calculated with current methods, our results shed light on the contribution of neogenesis to the dynamics...
of β-cell mass in adult animals. β-Cell number depends on the balance between β-cell replication, β-cell neogenesis, and β-cell death. Because all 3 parameters remained stable from month 7 to month 20, neogenesis was also stable in this period, even though we do not know its rate.

β-Cell mass increased with age, in agreement with previous studies (31–34). The classic work of Hellman (31) concentrated on the initial 100 days of life and included only one group of older rats; in contrast, our study allows a more detailed assessment of the evolution of β-cell mass in adult and aged rats. Overall, β-cell mass increased ~8 times between 1 and 20 months of age, and although the main increment took place in the first 7 months of life (when β-cell mass increased 5–6 times), β-cell mass continued to grow even in the oldest group. This increment in β-cell mass closely matched that of total body weight at any time point and was selective for β-cells because the growth of the endocrine non–β-cell mass and the exocrine pancreas were limited to the initial months of life. The tight linear relationship and the constant ratio between β-cell mass and body weight throughout life suggests that the adaptation of β-cell mass is essential to maintaining normoglycemia in physiological conditions.

We also analyzed the changes in β-cell mass in light of the results on β-cell replication, size, and number. It must be pointed out that our methodology overestimates individual β-cell area (19), and therefore the calculated β-cell number was lower than the real number. However, because the underestimation of β-cell number affected all groups, the comparison among them remained valid. The number of β-cells per pancreas increased in the initial months of life and remained stable after month 7. On the other hand, the size of individual β-cells increased from month 1 to 7, remained relatively stable until month 15 (in agreement with Hellman’s results [33]), and increased markedly in aged rats. Therefore, in the initial months of life, both β-cell hyperplasia and hypertrophy contributed to the rapid increment in β-cell mass; in contrast, in aged animals, the increment of β-cell mass relied on β-cell hypertrophy. It could be speculated that this may reflect a limitation in β-cell replication capacity in old animals, which would depend exclusively on β-cell hypertrophy to increase their β-cell mass. Aging has been associated with disturbances in islet function and pathological changes in islets (21,35,36). Alternatively, the stimulus to increase β-cell size in aged animals could be of a different nature (37) or too modest to elicit a detectable increment in β-cell replication.

In summary, we have found that although β-cell replication decreases progressively in the initial months of life, it remains stable thereafter and is maintained even in the latest periods of life. In contrast, β-cell apoptosis is low and stable from month 1 to month 20 of life. β-Cell mass increases throughout life in tight parallelism with the increment in body weight, but the contribution of β-cell hypertrophy and β-cell hyperplasia to changes in β-cell mass is different depending on the age of the animals. Both β-cell number and size increase in the initial months of life; in contrast, only β-cell size increases in old animals. This study provides a global perspective for understanding the dynamics of β-cell mass in young, adult, and aged animals.

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