Genetic Background Determines the Size and Structure of the Endocrine Pancreas

Troels Bock,[1,2] Bente Pakkenberg,[1] and Karsten Buschard[2]

Key parameters of the endocrine pancreas, such as islet number, islet mass, β-cell mass, and α-cell mass, were studied in different strains of inbred mice to investigate the impact of genetic background on the size and structure of the endocrine pancreas. Six mice from each of seven different strains of inbred mice were included in the study. For all parameters investigated, there was a pronounced interstrain variation. ANCOVA showed that only mouse strain was statistically significant as an explanatory parameter for the number of islets. Mouse strain, body weight, and pancreas mass reached statistical significance as explanatory parameters for the islet mass, with mouse strain as the most significant predictor. These data show that genetic background is the most important predictor of both the number of islets and total islet volume. We also conclude that inbred mice could be a valuable resource to identify the genes responsible for the size and structure of the endocrine pancreas. *Diabetes* 54:133–137, 2005

Knowledge of the growth mode and regulation of the growth of the endocrine pancreas, in particular the β-cell compartment, is important for our understanding of the pathogenesis for type 2 diabetes and for choosing strategies when attempting to cure diabetes by increasing the β-cell mass. The way in which the endocrine pancreas expands during physiological growth and in models with islet regeneration is under debate. It has been known for many years that mature β-cells have the ability to replicate (1), but, in addition, other sources for new β-cells have been suggested, such as cells in the epithelium of the ducts (2,3), intraislet stem cells (4), and pancreatic acinar cells (5).

Based on studies (6,7) in normal rats and *ob/ob* mice, we previously proposed that islet neogenesis does not occur in the intact rodent pancreas. These data are in agreement with the recent finding by genetic lineage analysis that replication of mature β-cells is overwhelmingly dominating and may even be the only way new β-cells are formed from postnatal week 6 and onwards in mice (8).

Therefore, an obvious question is what determines the total number of islets? Theoretical possibilities include that the number of islets depends on body weight, size of the pancreas, total islet mass, or genetic background. The aim of this study was to investigate if quantitative parameters of the endocrine pancreas, such as the total islet number and the total mass of islets, β-cells, and α-cells, show significant variation between different strains of inbred mice with the explicit purpose of acquiring information about the role genetic background plays with respect to the total number of pancreatic islets.

RESEARCH DESIGN AND METHODS

All mice were 8-week-old males purchased from Taconic M&B (Ry, Denmark). Six mice from each of the following strains were examined: C57BL/6JBom (B6), DBA/2JBom (DBA/2), BALB/cABom (BALB/c), NOD/Bom (NOD), 129S6/SvEVBom (129S6), C3H/HeNBom (C3H), and CBA/JBom (CBA).

Histology. Mice were killed by breathing carbon dioxide followed by cervical dislocation. The pancreata were removed in total, weighed, and immersed in fixative (10% buffered formalin, pH 7.4) for at least 48 h before being embedded into paraffin. Each pancreas was exhaustively sectioned using a section thickness of 5 μm, as depicted in Fig. 1. Briefly, a random number between 1 and 50 was read from a random number table, e.g., 10, and thereafter section number 10, 12, 13, 14, 60, 62, 63, 64, etc., were sampled on glass slides until the entire pancreas was sectioned. In this case, the sections 10, 60, 110, etc., were termed primary sections and sections 12, 62, 112, etc., reference sections. Sections 13, 63, 113, etc., and sections 14, 64, 114, etc., are two sets of sections through the entire pancreas sampled according to systematic uniform random sampling (7,9).

All primary and reference sections were stained by Mayer's hematoxylin and eosin. Sections 13, 63, 113, etc., were immunostained to visualize insulin using a guinea pig anti-insulin antibody (dilution 1:100; Dako, Glostrup, Denmark) and EnVisionAP (prediluted; Dako) as a secondary antibody. Antibody binding was visualized by Fast Red (SigmaFAST; Sigma, Copenhagen, Denmark), and the sections were counterstained by Mayer's hematoxylin. Sections 14, 64, 114, etc., were immunostained to visualize glucagon using a rabbit anti-glucagon antibody (Dako; dilution 1:100) and EnVisionAP (Dako; prediluted) as a secondary antibody. Antibody binding was visualized by Fast Red (SigmaFAST; Sigma), and the sections were counterstained by Mayer's hematoxylin.

Stereology

Total mass of β-cells, α-cells, islets, and pancreas. Total β-cell, α-cell, and islet mass were determined as previously described (10). Briefly, using the measurement of total β-cell mass as an example, the sections were investigated using a microscope (Leica DMLB; Leica, Herlev, Denmark) equipped with a projecting arm (Leica) to project the image onto the table and with a microscope stage connected to an xy stepper (ECO-drive; Märzhäuser Wetzlar, Wetzlar, Germany) controlled by a computer using the Win-Commander 4.1.3.0. software (Märzhäuser). The final magnification was ×170. All insulin-stained sections from each pancreas (sections 13, 63, 113, etc.) were then systematically investigated according to systematic uniform random sampling (10) starting at a random position outside the sections and then moving the stage "meander-like" with fixed step lengths in the x and y directions through all sampled sections from each pancreas. A point-counting grid with 99 points, 1 of them encircled (the unit point), was attached to the table, and for each pancreas the total number of grid points that hit β-cells and the total number of unit points that hit the pancreas or nonpancreatic tissue, such as fat or

From the [1]Research Laboratory for Stereology and Neuroscience, Hovedstadens Sygehusfællesskab Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark; and the [2]Hovedstadens Sygehusfællesskab Bartholin Institute, Copenhagen University Hospital, Copenhagen, Denmark.

Address correspondence and reprint requests to Troels Bock, MD, PhD, Research Laboratory for Stereology and Neuroscience, H:S Bispebjerg Hospital, Copenhagen University Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark. E-mail: tbock@post12.tele.dk.

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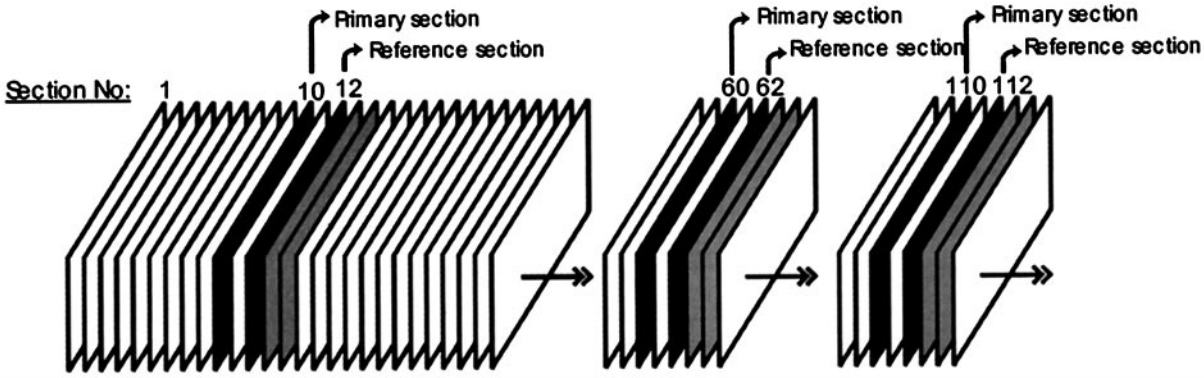

FIG. 1. Sectioning and sampling of sections from pancreas. Refer to text for further explanations.

lymphoid tissue, were counted. The total β-cell mass for each pancreas was then calculated by:

$$M_\beta = \frac{P_\beta}{99 \times (P_{U\text{-}pan} + P_{U\text{-}nonpan})} \times M_{tis}$$

where M_β is the total β-cell mass, P_β is total number of grid points that hit β-cells in all investigated sections from one pancreas, $P_{U\text{-}pan}$ is the number of unit points that hit the pancreas, $P_{U\text{-}nonpan}$ is the number of unit points that hit nonpancreatic tissue, and M_{tis} is the wet weight of the removed tissue.

The total α-cell and islet mass were determined in parallel with the method described above using the sections stained for glucagon (sections 14, 64, 114, etc.) and the hematoxylin and eosin–stained primary sections (sections 10, 60, 110, etc.), respectively.

The total mass of pancreas was calculated by:

$$M_{pan} = \frac{P_{U\text{-}pan}}{P_{U\text{-}pan} + P_{U\text{-}nonpan}} \times M_{tis}$$

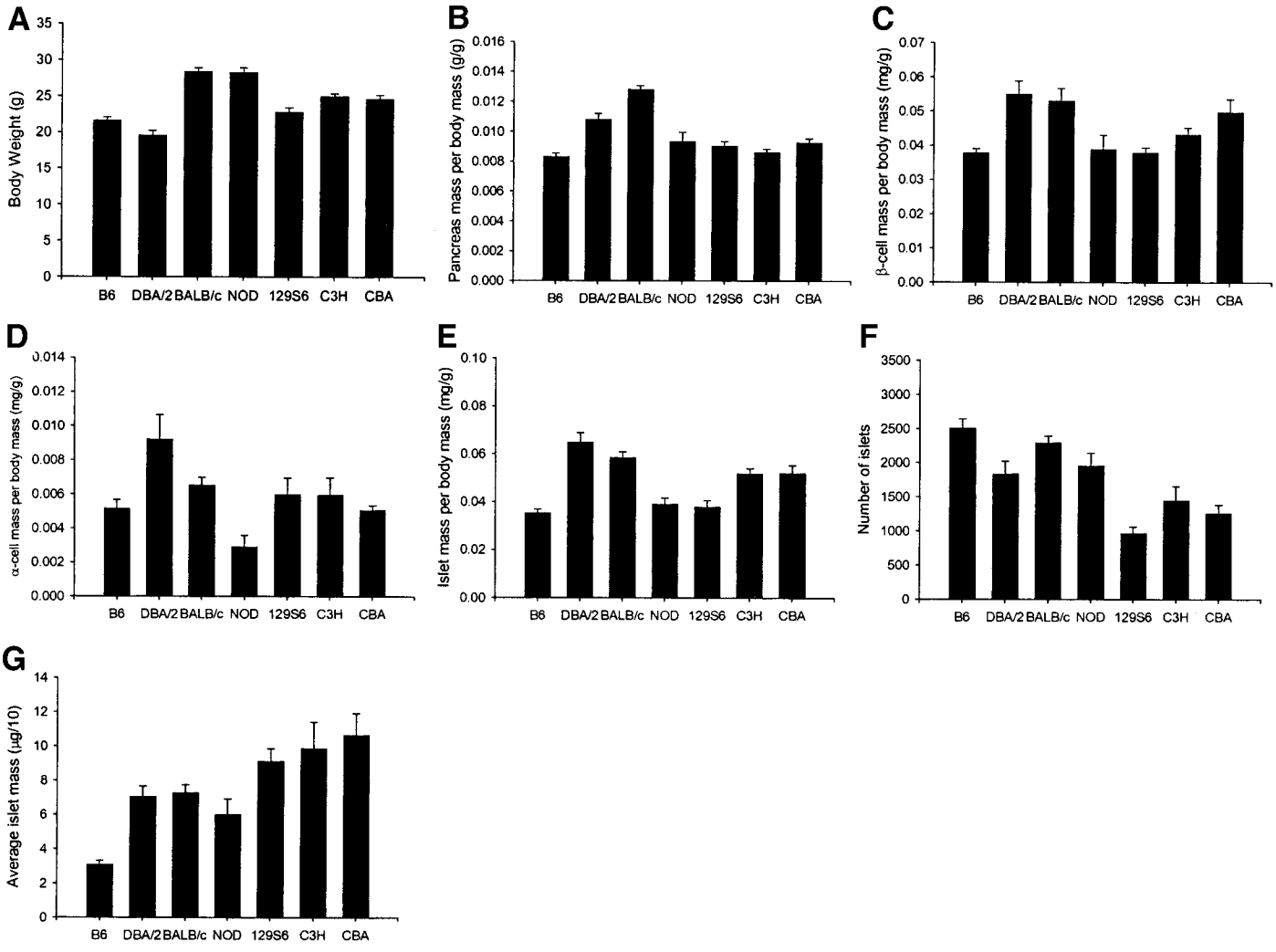

FIG. 2. The mean values for the investigated parameters in the different mouse strains. Error bars represent the SEs in all plots. Parameters investigated are body mass (*A*), pancreas mass per body mass (*B*), β-cell mass per body mass (*C*), α-cell mass per body mass (*D*), islet mass per body mass (*E*), total number of islets (*F*), and average islet mass (*G*). All variables showed significant variation between the mouse strains ($P < 0.001$ for each parameter). Differences between the single strains are evaluated in Table 1.

where M_{pan} is the pancreas mass and the other abbreviations are as defined above.

Total number of islets and mean islet mass. The total number of islets was determined as previously described by the so-called physical fractionator method (7,11). Briefly, all primary and reference sections from each pancreas were investigated using two identical microscopes (Leica DMLB; Leica), both equipped with projecting arms, one of them with a computer-controlled stage, and the other with a manual stage. An unbiased sampling frame was attached to the table with the microscope that had the computer-controlled stage. All primary sections were investigated systematically according to systematic uniform random sampling, and for each islet sampled in the sampling frame, it was determined in the other microscope whether the islet also appeared in the reference section. The definition of an islet was chosen as a cluster of cells with a minimum of four visible nuclei displaying the typical characteristics of islet cells with pale cytoplasm and approximately spherical nuclei. A detailed discussion of why hematoxylin and eosin–stained sections are preferable for this method has previously been given (7). The total number of islets was finally calculated by:

$$N_{isl} = \frac{N_{sect(p\text{-}p)}}{N_{sect(p\text{-}r)}} \times \frac{\Delta x \times \Delta y}{A_{frame}} \times \Sigma\, Q^{-}_{isl}$$

where $N_{sect(p\text{-}p)}$ is the number of sections between the primary sections (50 in this case), $N_{sect(p\text{-}r)}$ is the number of sections between a primary section and the corresponding reference section (2 in this case), Δx is the step length in the x direction, Δy is the step length in the y direction, A_{frame} is the area of the sampling frame corrected for magnification, and $\Sigma\, Q^{-}_{isl}$ is the total number of islets counted (sampled in the primary section but absent in the reference section) from one pancreas.

The mean islet mass was then calculated by:

$$m_{isl} = \frac{M_{isl}}{N_{isl}}$$

where m_{isl} is the mean islet mass.

Statistics. Data are presented as means ± SE for each parameter investigated. The overall difference between strains was evaluated by ANOVA (SigmaStat 2.0; SPSS, Chicago, IL). Student-Newman-Keuls multiple range test (SigmaStat 2.0) was used to evaluate differences between the single strains if an overall significant difference between the strains was found. The Pearson product moment (SigmaStat 2.0) method was used to evaluate for significant correlations. ANCOVA was performed using the SAS 8.2 software (SAS Institute, Copenhagen, Denmark). The level of significance was set to $P < 0.05$.

RESULTS

Figure 2 shows the results of the stereological investigations for the seven different strains of inbred mice. For all investigated parameters, ANOVA showed a statistically significant variation between the mouse strains of $P < 0.001$ for each parameter investigated. The results of the Student-Newman-Keuls multiple range tests (all pairwise) are shown in Table 1. B6 mice had the lowest mass per body weight of both islets and β-cells, e.g., only 55% of the islet mass per body weight found in DBA/2 mice ($P < 0.001$). In contrast, B6 mice had the highest number of islets among the strains, visualizing the lack of a positive association between the number of islets and the total islet mass. There was an especially pronounced interstrain variation in the total islet number and the mean islet mass. As examples, 129S6 mice had a mean of 971 ± 88 islets per pancreas compared with 2,509 ± 133 islets per pancreas in B6 mice ($P < 0.001$), whereas B6 mice had a mean islet mass of 3.09 ± 0.21 μg/10 compared with 10.66 ± 1.25 μg/10 in CBA mice ($P < 0.001$).

Figure 3*A* shows plots of the total islet mass versus body weight, the total pancreas mass, and the total number of islets for all mice irrespective of strain. There was a strong correlation between the islet mass and body weight and between islet and pancreas mass, while the number of islets and the islet mass were not significantly correlated. Figure 3*B* shows that the total number of islets was not significantly correlated with body weight, pancreas mass, or total islet mass.

TABLE 1
Results of the all-pairwise comparisons of investigated parameters by Student-Newman-Keuls multiple range test

Parameter	Significant differences between strains
Body weight	BALB/c > B6*, DBA/2*, 129S6*, C3H*, CBA*
	NOD > B6*, DBA/2*, 129S6*, C3H*, CBA*
	C3H > B6†, DBA/2*, 129S6‡
	CBA > B6†, DBA/2*, 129S6‡
	129S6 > DBA/2*
	B6 > DBA/2‡
Pancreas mass per body mass	BALB/c > B6*, DBA/2*, NOD*, 129S6*, C3H*, CBA*
	DBA/2 > B6*, NOD†, 129S6†, C3H*, CBA†
β-Cell mass per body mass	DBA/2 > B6†, NOD†, 129S6†, C3H‡
	BALB/c > B6‡, NOD‡, C3H‡
α-Cell mass per body mass	DBA/2 > B6‡, BALB/c‡, NOD*, 129S6‡, C3H‡, CBA‡
	BALB/c > NOD‡
Islet mass per body mass	DBA/2 > B6*, NOD*, 129S6*, C3H†, CBA†
	BALB/c > B6*, NOD*, 129S6*
	CBA > B6*, NOD†, 129S6†
	C3H > B6*, NOD†, 129S6†
Total islet number	B6 > DBA/2‡ NOD‡, 129S6*, C3H*, CBA*
	BALB/c > 129S6*, C3H†, CBA*
	NOD > 129S6*, CBA‡
	DBA/2 > 129S6*, CBA‡
Mean islet mass	CBA > B6*, NOD‡
	C3H > B6*, NOD‡
	129S6 > B6*
	BALB/c > B6‡
	DBA/2 > B6‡
	NOD > B6‡

*$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$.

Based on these data, we performed an ANCOVA in a linear model, using the number of islets as the dependent variable and the parameters mouse strain, total pancreas and total islet mass, and body weight as explanatory variables. Of these parameters, only the mouse strain ($P < 0.0001$) was left in the model since none of the other parameters were statistically significant ($P > 0.05$ for each of the other three parameters). The model with mouse strain as the single independent parameter showed a coefficient of determination (R^2) of 0.70, underlining the importance of the genetic background for the number of pancreatic islets.

ANCOVA using the total islet mass as an independent parameter and the mouse strain, the total pancreas mass, the number of islets, and the body weight as explanatory parameters showed that only the number of islets did not reach statistical significance ($P > 0.05$) and was therefore excluded from the model, whereas the parameters body weight ($P = 0.029$), pancreas mass ($P = 0.004$), and mouse strain ($P < 0.0001$) were all included in the model that gave a coefficient of determination of 0.86.

DISCUSSION

This study demonstrates pronounced interstrain variation in quantitative measures of the endocrine pancreas. No other previous study has directly compared a broad range of mouse strains in the same investigation. Due to the con-

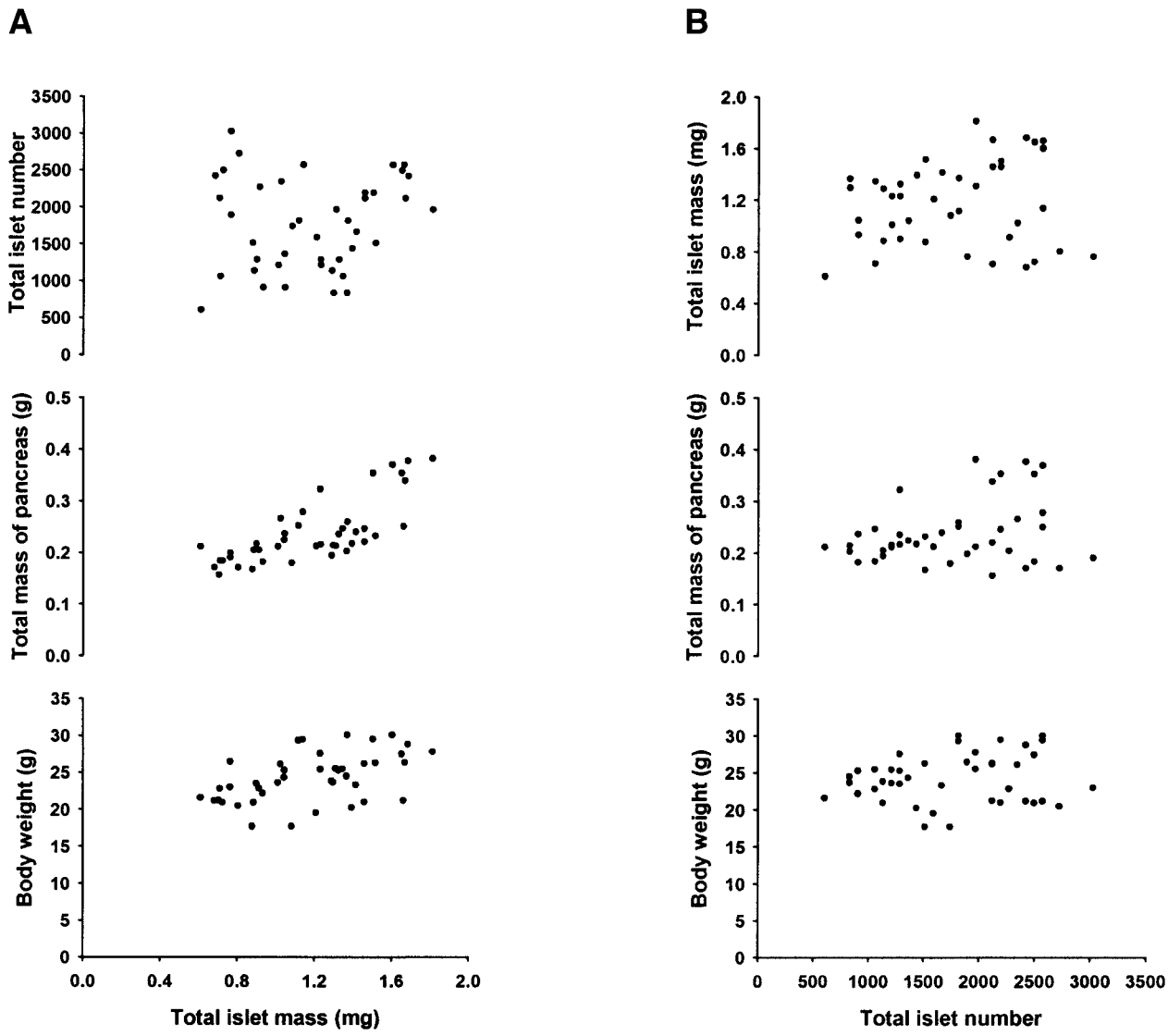

FIG. 3. Plots of data from all 42 mice in the study, irrespective of mouse strain. *A*: Body mass, total pancreas mass, and total islet number as a function of the total islet mass. There was a significant correlation between the total islet mass and the body mass ($P < 0.001$) and between the total islet mass and the total pancreas mass ($P < 0.001$), whereas no significant correlation was found between the total islet mass and the total islet number ($P = 0.37$). *B*: Body mass, total pancreas mass, and total islet mass as a function of total islet number. No significant correlations were found between the islet number and any of these parameters ($P > 0.05$ for each parameter).

siderable variation in methods previously used to quantify the endocrine pancreas, it would be difficult and fraught with uncertainty to extract similar data from previous studies retrospectively.

The data show that genetic factors are major determinants for parameters such as the total number of islets and the total islet mass. The number of islets seems especially to be under tight genetic control, since *1*) there was a pronounced variation in the islet number between the strains investigated, *2*) no significant correlation was found between the total islet number and either the body mass, total islet mass, or the total pancreas mass, and *3*) ANCOVA showed that among the parameters investigated, only mouse strain was significantly included in a linear model with the number of islets as the dependent parameter, and a model based on that parameter alone gave a coefficient of determination of 70%.

We previously reported that *ob/ob* mice compared with lean ob/+ mice do not have an increased number of islets despite a 3.6-fold increase in the total islet mass, and we proposed from this study (7) and previous findings (6) in rats that the source of new islet cells in rodents after a certain age is cells already found within existing islets. In addition, it was recently shown by genetic lineage tracing that β-cells in adult mice are formed by replication of existing β-cells, both during physiological growth and after partial pancreatectomy (8). Thus, it now seems to be established that in mice during physiological growth from week 6 and onwards, after partial pancreatectomy and in expansion of the β-cell number in *ob/ob* mice, the contribution to the number of new β-cells from other sources than replication of mature β-cells is negligible. This is apparently in contrast to other theories suggesting that other cell types such as ductal cells (2), acinar cells (5), and intraislet non–β-cells (4) can also contribute to the maintenance and expansion of the β-cell mass. If the growth of the total islet mass included formation of new islets, then a positive correlation between the total islet

number and the total islet mass would have been expected, at least in mice from the same strain. Such a relationship was not found in the present study, since the parameter total islet mass did not reach significance in the ANCOVA with the total islet number as the independent parameter. Thus, our data are in agreement with what would have been expected according to the theory of a fixed islet number from a given (and yet undefined) age. However, this is only indirect evidence for this theory, as our investigation did not include mice at different ages.

The total islet mass was correlated with both the body weight and the pancreas mass, besides showing a significant variation between strains. The coefficient of determination in a linear model including mouse strain, pancreas mass, and body weight as explanatory parameters for the total islet mass was 0.86, showing that the vast majority of the variation was accounted for in the model with these three explanatory variables, among which mouse strain was the most significant single variable. Thus, even though there is a strong hereditary component, environmental factors such as acquired body weight can also influence the total islet mass. The data for the β-cell mass per body mass showed the same overall pattern as for the islet mass per body mass, which is not surprising since β-cells are the most frequent cell type within the islets.

These findings open up an obvious possibility for identification of the genes that are responsible for the size and structure of the endocrine part of the pancreas (12). However, that genetic factors are important for, e.g., the β-cell mass, does not necessarily imply the existence of genes that directly control the β-cell mass by regulating features such as tendencies for β-cell mitosis or apoptosis, even though the existence of such genes certainly cannot be excluded. That possibility is supported by the previous finding that islets from C57BL/6J and C57BL/KsJ mice differ markedly in their glucose-stimulated islet cell replication both in vivo and in vitro (13). In addition, the genetic background is important for the phenotypic outcome of several models of genetically induced diabetes such as the Lep^{ob} and $Lepr^{db}$ mutations (14), mice double heterozygous for knockout of the insulin receptor and insulin receptor substrate-1 (15), as well as chemically induced diabetes (16). Another possibility is that the observed differences in, e.g., the β-cell mass, reflect compensatory effects due to genetically determined differences in processes important for the need for insulin, such as insulin sensitivity or gluconeogenesis. Indeed, pronounced differences between mouse strains with respect to, e.g., insulin and glucose tolerance tests, have recently been described by Goren et al. (17). Their investigation included a detailed evaluation of glucose homeostasis in 8-week-old male B6 mice and DBA/2 mice, i.e., mice similar to two of the groups in this study. Among their findings were that B6 mice had significantly higher fasting and fed blood glucose concentrations as well as a higher glucose excursion after an intraperitoneal glucose load compared with DBA/2 mice. This is interesting since B6 mice had the lowest and DBA/2 mice the highest islet and β-cell mass per body weight in this study, suggesting a relationship between glucose homeostasis and size of the endocrine pancreas also in the nondiabetic state. Meanwhile, which of these strain-dependent differences in tests of glucose homeostasis and size and structure of the endocrine pancreas that are primary and secondary to each other, and whether they are hierarchically related at all, cannot be determined from this investigation. This issue should, however, be addressed in cross-breeding studies.

In conclusion, we have demonstrated a pronounced variation in islet mass, β-cell mass, α-cell mass, mean islet mass, and islet number between different strains of inbred mice, and the number of pancreatic islets seems especially to be under tight genetic control. These results will enable identification of genes determining the size and structure of the endocrine pancreas.

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