Increased Islet Volume but Unchanged Islet Number in *ob/ob* Mice

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It is important for our understanding of the pancreatic islets to study whether new islets are able to form in the intact pancreas. We developed a new method to determine the total number and the mean volume of the pancreatic islets, and we used this method to study the expansion of the islet mass in *ob/ob* mice ($n = 8$), using *ob/+* mice ($n = 8$) as controls. The total islet volume was increased by a factor of 3.6 in *ob/ob* mice compared with *ob/+* mice, whereas, importantly, the total number of islets did not differ among *ob/ob* mice and *ob/+* mice (3,193 ± 160 islets in *ob/ob* mice vs. 3,184 ± 142 islets in *ob/+* mice, $P = 0.97$). The coefficient of variation in the volume distribution of islets was equal in the two groups, showing that in *ob/ob* mice, the existing islets expand their volume by the same proportion, without a net formation of new islets. We suggest that the pancreatic islets should be considered as anatomically such complex structures that islet neogenesis does not spontaneously occur in an intact pancreas. Cells within the existing islets are presumably the most important sources for islet cell hyperplasia during expansion of the total islet mass. *Diabetes* 52:1716–1722, 2003

A fundamental and yet unanswered question in the understanding of the pathogenesis of type 2 diabetes is the limitation in the formation of enough additional β-cells to maintain normoglycemia during an increased demand for insulin; even individuals with significant insulin resistance do not develop diabetes if they have an appropriate number of functioning β-cells, emphasizing the pathophysiological importance of the total β-cell mass.

The *ob/ob* mouse has been extensively studied as a model of type 2 diabetes (1). These mice are obese and develop insulin resistance and diabetes because of an inherited inability to produce leptin (2), despite a marked expansion of the total mass of the pancreatic islets. It has been stated in the literature that both islet hyperplasia and islet hypertrophia are responsible for the increase in the total islet mass in *ob/ob* mice (3,4), but among the few studies that actually addressed this question by applying methods that, in theory, could measure the total number of islets (5,6), the results are in fact diverging. Previously, we found a linear correlation between the total islet volume and the volume-weighted mean volume in rats (7), a result that suggested—but, because of the nature of the method, could not prove—that the expansion of the islet mass during physiological growth in rats is caused by the growth of existing islets without the formation of new islets. We therefore developed a method relying on recent stereological methods to determine the total number and mean volume of the pancreatic islets, and we used this method to investigate the expansion of the islet mass in *ob/ob* mice. An understanding of whether new islets are able to form in the intact pancreas is important for understanding the islets as an anatomical structure, and, in addition, it could be important for understanding which cells within the pancreas are important for the expansion of the total islet mass during physiological or pathological conditions.

RESEARCH DESIGN AND METHODS

Animals. Eight male C57BL/6JBom-*ob/ob* and eight male C57BL/6Jbom-*ob/+* mice were purchased (M&B, Ry, Denmark). All mice were 8 weeks old. Fasting blood glucose was measured by a Glucometer Elite (Bayer, Leverkusen, Germany) before the mice were killed by cervical dislocation. The pancreas was immediately removed and fixed in 10% buffered formaline, pH 7.4.

Histology and sampling of sections. Each pancreas was embedded in paraffin and sectioned exhaustively into 5-μm-thick sections. Figure 1 illustrates the sampling of sections. With a random start between the first 50 sections, every 50th section was sampled (the primary sections). In addition, for every sampled primary section, the section two sections ahead was sampled as the reference section. Because every section was 5 μm thick, it follows that there was 250 μm between the primary sections and 10 μm between a primary section and the corresponding reference section. All primary and reference sections were stained with hematoxylin and eosin (H&E). For four pancreata in each group, an additional set of systematically sampled sections were stained for insulin by incubation with a primary guinea pig anti-swine insulin antibody (dilution 1:50; Dako, Glostrup, Denmark), followed by incubation with Envision-AP (Dako) and subsequently visualized by a Sigma-FAST Fast Red system (Sigma, Vallensbaek, Denmark) and counterstained by hematoxylin.

Microscopes and equipment. The sections were investigated by a Leica DMLB microscope (Leica, Glostrup, Denmark) equipped with a projecting arm to project the image onto the table. Systematic uniform random sampling within a section was enabled by using a ECO-Drive microscope stage (Märzhäuser Wetzlar, Wetzlar, Germany) controlled by a computer with WIN-Commander 4.1.3.0 software (Märzhäuser). The applied probes used for the stereological examinations (point-counting grid or counting frame as described below) were fixed to the table so that the microscope projected the image onto the grid. For counting the total number of islets, we used an additional identical microscope with a projecting arm but with a manual microscope stage.

Total volumes of islets and pancreas. Using step-lengths of 900 μm in the x-direction (Δx) and 700 μm in the y-direction (Δy), all primary sections from each pancreas were systematically examined as illustrated in Fig. 2. A

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BrdU, bromodeoxyuridine; H&E, hematoxylin and eosin; PDX-1, pancreas duodenum homeobox-1.

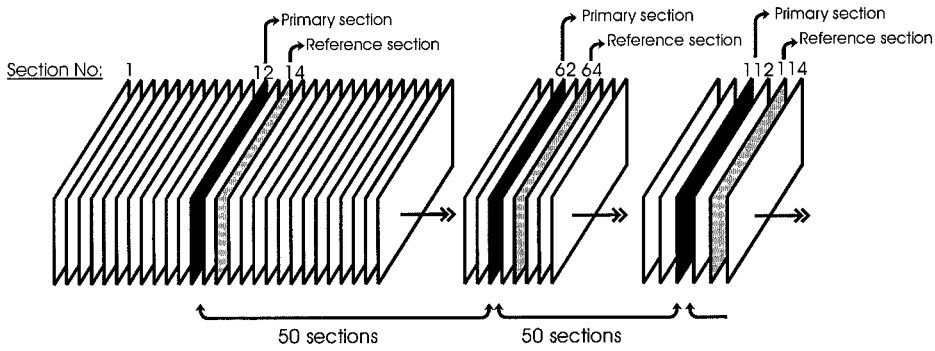

FIG. 1. The figure illustrates the sampling of histological sections. The pancreas was exhaustively sectioned, and both the sections marked as primary and the reference sections were used in the stereological investigations.

point-counting grid with 99 points, 1 of them encircled, was applied. Moving through all primary sections from the pancreata as illustrated in Fig. 2, we counted how many times 1 of the 99 points hit an islet. An islet was defined as a cluster of cells with a minimum of three visible nuclei displaying the normal characteristics of islet endocrine cells (pale cytoplasm with approximately spherical nuclei). Simultaneously, we counted how many times the encircled point hit pancreatic tissue (exocrine pancreatic tissue, ducts, vessels, islets, etc.). The values for the total volume of pancreas and the islets of Langerhans were then calculated based on the Cavalieri principle (8):

$$V(\text{pan}) = a/p\,(\text{pan}) \times N_{\text{sect}}(p - p) \times T_{\text{sect}} \times \sum P(\text{pan}) = 0.1575\ \text{mm}^3 \times \sum P(\text{pan}) \quad (1)$$

where V(pan) is the total volume of pancreas, a/p(pan) is the area per point (in this case $\Delta x \times \Delta y$ because only one point in the grid was used to count points that hit pancreas), $N_{\text{sect}}(p - p)$ is the number of sections between the primary sections (50 sections in this case), T_{sect} is the section thickness (5 μm), and ΣP(pan) is the total number of points that hit pancreas.

$$V(\text{isl}) = a/p\,(\text{isl}) \times N_{\text{sect}}(p - p) \times T_{\text{sect}} \times \sum P(\text{isl}) = 1.591 \times 10^{-3}\ \text{mm}^3 \times \sum P(\text{isl}) \quad (2)$$

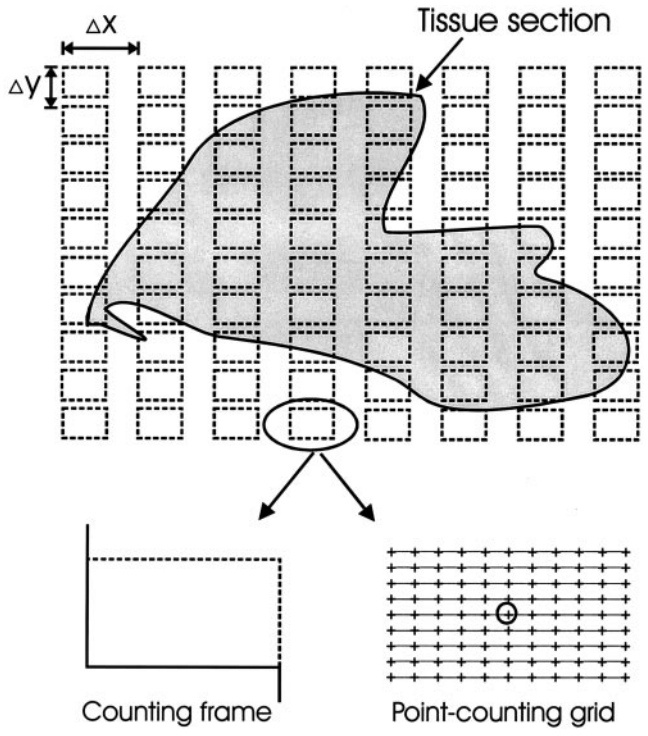

FIG. 2. Each sampled primary section was systematically investigated as illustrated in the top of the figure. This was performed in two sessions during which either the counting frame (lower left in the figure) was attached to the table to count the islets as described in the text, or the point-counting grid (lower right in the figure) was attached to the table to count the total volume of islets, the total volume of pancreas, and the volume-weighted mean islet volume as described in the text.

where V(isl) is the total volume of islets, a/p(isl) is the area per point (in this case $\Delta x \times \Delta y/99$ because there were 99 points in the grid used to count points that hit islets), and ΣP(isl) is the total number of points that hit the islets.

Volume-weighted mean islet volume. For each point that hit an islet, we also measured the distance in the horizontal direction between the intercepts of a horizontal line through the point and the islet. A detailed theoretical and practical description of the volume-weighted mean islet volume as a stereological parameter has previously been published (7). Briefly, the volume-weighted mean islet volume is the mean volume if islets are weighted proportionally to their volume. Unless all islets have identical volumes, then the volume-weighted mean islet volume is higher than the number-weighted mean islet volume (the "ordinary" mean islet volume). The volume-weighted mean islet volume of the islets could then be calculated according to:

$$v_V = \frac{\pi}{3} \times \overline{l_0^3} \quad (3)$$

where v_v is the volume-weighted mean islet volume, and l_0 is the length of the line between the two intercepts of an islet and a horizontal line through a point that hits the islet.

Total number of islets. In another session, the sampling within the primary sections was performed as described above and depicted in Fig. 2, but an unbiased counting frame (lower left in Fig. 2) was now attached to the table. The rules of the counting frame define objects completely outside the frame or objects that touch the exclusion lines (the full lines in the figure) as being outside the frame, whereas objects that are completely within the frame or touch only the inclusion lines (the dashed lines in the figure) are defined as being within the frame. We applied the disector principle (9) to count the islets as depicted in Fig. 3. Whenever an islet profile was sampled by the counting frame, the corresponding position in the reference section was located with the other microscope, and we determined whether the islet was also visible in the reference section. An islet was counted if it appeared in the primary section but not in the reference section (islet C in Fig. 3). Because the sampling of sections as well as the within-section sampling were performed with known sampling fractions, the total number of islets can be calculated according to the fractionator principle (10) from:

$$N(\text{isl}) = \frac{N_{\text{sect}}(p - p)}{N_{\text{sect}}(p - r)} \times \frac{\Delta x \times \Delta y}{A(\text{frame})} \times \sum Q^-(\text{isl}) = 34.94 \times \sum Q^-(\text{isl}) \quad (4)$$

where N(isl) is the total number of islets in the pancreas, $N_{\text{sect}}(p - p)$ is the number of sections between the primary sections, $N_{\text{sect}}(p - r)$ is the number of sections between a primary section and the corresponding reference section (two in this case), Δx and Δy are the step lengths, A(frame) is the area of the counting frame corrected for magnification (450,736 μm^2), and ΣQ^-(isl) is the total number of islets counted in one pancreas.

Number-weighted mean islet volume. The number-weighted mean islet volume (the "ordinary" mean islet volume) was calculated from:

$$v_N(\text{isl}) = \frac{V(\text{isl})}{N(\text{isl})} = \frac{1.591 \times 10^{-3}\ \text{mm}^3 \times \sum P(\text{isl})}{34.94 \times \sum Q^-(\text{isl})} = 4.554 \times 10^4\ \mu\text{m}^3 \times \frac{\sum P(\text{isl})}{\sum Q^-(\text{isl})} \quad (5)$$

where v_N(isl) is the mean islet volume (the index N is to stress that it is the "ordinary" or number-weighted mean volume), and the other abbreviations are as explained in the paragraphs above.

The variation in the volume distribution of islets. Knowing both the number-weighted mean islet volume and the volume-weighted mean islet volume enables a calculation of the coefficient of variation (the standard

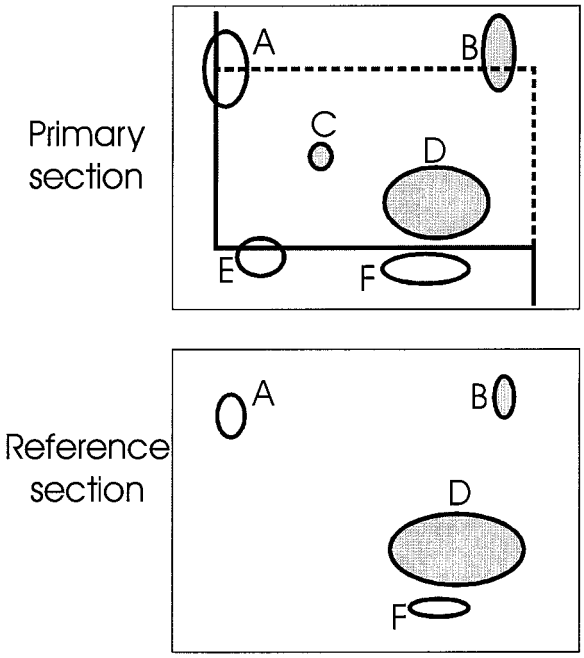

FIG. 3. An example of disector counting. In the primary section, islets denoted B, C, and D are defined as within the counting frame because they do not touch the (full) exclusion lines, and they are either completely within the frame or touch the (dashed) inclusion lines. The islets denoted A, E, and F are defined as being outside the frame because they are either completely outside the frame or touch the exclusion lines. In the reference section shown at the bottom of the figure, islets B and D are still present, and therefore they are not counted, whereas islet C is not present, and therefore it is counted (see text for more details).

deviation divided by the mean) in the volume distribution of islets in pancreas without assumptions of the type of distribution (7) according to:

$$CV_N(isl) = \sqrt{\frac{\overline{v_V(isl)}}{\overline{v_N(isl)}} - 1} \quad (6)$$

where $CV_N(isl)$ is the coefficient of variation in the distribution of islet volumes.

Number of islet profiles per area of pancreas and mean islet area. When the examination of sections was performed with the counting frame attached to the table, we also counted the number of islet profiles that were sampled in the counting frames (islets B, C, and D in Fig. 3). The number of islet profiles per area of pancreas was then calculated from:

$$N_{prf,A}(isl,pan) = \frac{\left(\frac{\Delta x \times \Delta y}{A(frame)} \times \sum N_{prf}(isl)\right)}{\left(a/p(pan) \times \sum P(pan)\right)} = \frac{1}{450{,}736\ \mu m^2} \times \frac{\sum N_{prf}(isl)}{\sum P(pan)} \quad (7)$$

where $N_{prf,A}(isl,pan)$ is the number of islet profiles per area of pancreas, $\Sigma N_{prf}(isl)$ is the total number of islet profiles counted in the counting frames in all primary sections from one pancreas, and the remaining factors are as defined in the previous paragraphs.

The mean islet area was calculated from:

$$\overline{a_{prf}(isl)} = \frac{\left(a/p(isl) \times \sum P(isl)\right)}{\left(\frac{\Delta x \times \Delta y}{A(frame) \times \sum N_{prf}(isl)}\right)} = 4{,}553\ \mu m^2 \times \frac{\sum P(isl)}{\sum N_{prf}(isl)} \quad (8)$$

where $a_{prf}(isl)$ is the mean islet profile area, and the remaining factors are as defined in the previous paragraphs.

In some previous studies, the number of islet profiles per area of pancreas has been treated as an indicator of the total number of islets, and, in parallel, the mean islet profile area has been treated as an indicator of the mean islet volume. As shown below, none of these approaches can be theoretically validated. In a previous publication (7), we have shown that the number of islet profiles per area of pancreas and the mean islet area can, using the formula originally given by Abercrombie (11), be expressed as:

$$N_{prf,A}(isl,pan) = \frac{N(isl) \times (h + T_{sect})}{v(pan)} \quad (9)$$

$$a_{prf}(isl) = \frac{v_N(isl)}{(h + T_{sect})} \quad (10)$$

where $N_{prf,A}(isl,pan)$ is the number of islet profiles per area of pancreas, N(isl) is the total number of islets in the pancreas, h is the mean extension of the islets measured perpendicular to the sectioning direction (the mean islet height), T_{sect} is the section thickness, V(pan) is the total volume of pancreas, $a_{prf}(isl)$ is the mean islet profile area, and $v_N(isl)$ is the mean islet volume.

Equation 9 shows that the number of profiles per area of pancreas in one or several sections is not a reliable reflector of changes in the total number of islets because it, besides the total number of islets, also depends on the volume of pancreas, the mean height of the islets, and the section thickness. As seen from Eq. 10, the mean islet profile area is directly proportional with the mean islet volume but inversely proportional to the sum of the mean height of the islets and the section thickness. This disqualifies this parameter as a reliable reflector of the absolute value or changes in the mean islet volume. Nevertheless, to enable a comparison between the conclusions reached using the theoretically validated set of stereological methods described above and the conclusions that would have been reached if the conclusions had been based on measurements of the number of profiles per area of pancreas and the mean islet area, we also measured the latter two parameters in this study.

The consequence of ignoring single endocrine cells. How many endocrine pancreatic cells should be clustered together before the term islet of Langerhans is appropriate has not yet been defined, nor is it relevant from a clinical perspective. In this study, we used H&E staining to identify islets, and we defined an islet profile as a cluster of endocrine cells with at least three visible nuclei. To get an impression of the fraction of β-cells ignored due to the choice of staining method, we used a set of sections stained for insulin from four *ob/ob* mice and four *ob/+* mice. Using a point-counting grid with 99 points and sampling as described above, we counted the number of points that hit β-cells within islets according to the definition used for the H&E-stained sections, and we counted the number of points that hit β-cells that were not within clusters defined as islets. For *ob/ob* mice, the mean fraction of points that hit β-cells not within islets was 1.3%, whereas the corresponding value for *ob/+* mice was 1.7%, illustrating that the β-cells not considered in the measured parameters in this study represent a very small fraction of the total islet volume.

Statistics. Data in figures are presented as the means ± SE. The results from *ob/ob* mice and *ob/+* mice were compared by Student's *t* test. All *P* values are two-sided, and the level of significance was set to $P < 0.05$.

RESULTS

As expected, mean body weight and mean blood glucose were significantly higher in *ob/ob* mice compared with *ob/+* mice (Fig. 4*A* and *B*), whereas the total volume of pancreas (Fig. 4*C*) was equal in the two groups. The total islet volume (Fig. 4*D*) was 3.67 times higher in *ob/ob* mice (2.09 ± 0.19 mm^3 in *ob/ob* mice vs. 0.57 ± 0.03 mm^3 in *ob/+* mice, $P < 10^{-5}$). As seen from Fig. 4*E*, the total number of islets was strikingly equal in the two groups ($3{,}193 \pm 160$ in *ob/ob* mice and $3{,}184 \pm 142$ in *ob/+* mice, $P = 0.97$), and the 95% CI ranged from +468 to −450 islets in *ob/ob* mice compared with *ob/+* mice. The ordinary mean islet volume (Fig. 4*F*) was $6.57 \pm 0.52 \times 10^5$ μm^3 in *ob/ob* mice vs. $1.81 \pm 0.12 \times 10^5$ μm^3 in *ob/+* mice ($P < 10^{-6}$). Thus, the pronounced increased total islet volume in *ob/ob* mice was caused solely by islet hypertrophy, with no contribution from a net formation of new islets.

The volume-weighted mean islet volume (Fig. 4*G*) was $2.00 \pm 0.25 \times 10^7$ μm^3 in *ob/ob* mice vs. $0.52 \pm 0.05 \times 10^7$ μm^3 in *ob/+* mice ($P < 10^{-4}$). In other words, the volume-weighted mean islet volume and the number-weighted mean islet volume were increased in *ob/ob* mice by approximately the same factor (3.88 for the volume-weighted mean islet volume and 3.63 for the number-

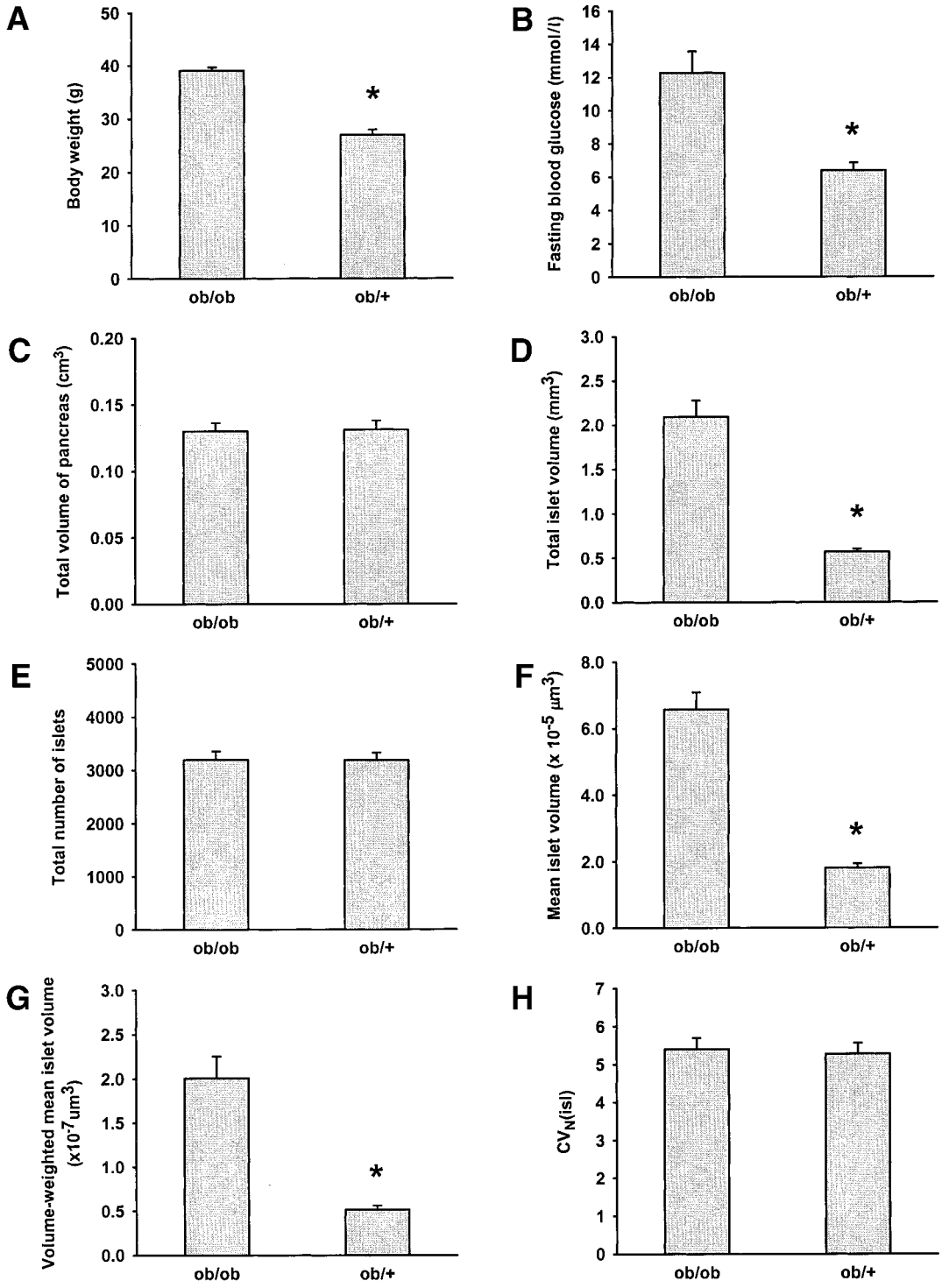

FIG. 4. The results from the stereological investigation of the pancreata. Data are presented as group means, with the error bars representing the SE. *A*: Body weight. *B*: Fasting blood glucose. *C*: Total volume of pancreas. *D*: Total islet volume. *E*: Total number of islets. *F*: Mean islet volume. *G*: Volume-weighted mean islet volume. *H*: CV_N(isl). *Statistically significant difference between the *ob/ob* group and the *ob/+* group. The actual *P* values are given in the text.

weighted mean islet volume). As a consequence (Eq. 6), the coefficient of variation in the distribution of islet volumes (Fig. 4*H*) was similar in the two groups (5.39 ± 0.29 in *ob/ob* mice vs. 5.27 ± 0.29 in *ob/+* mice, $P = 0.76$). This shows that not only is the number of islets unchanged in *ob/ob* mice, but also the shape of the distribution of islet volumes seems to be identical in the two groups. Thus, the stereological investigation showed that the increased total islet volume found in *ob/ob* mice was a result of islet hypertrophia, with no detectable contribution from islet hyperplasia, and the islet hypertrophia could be explained as if, in theory, all islets increased their volume by the same factor.

The number of islets per area of pancreas (Fig. 5*A*) was 1.52 ± 0.10 mm^{-1} in *ob/ob* mice and 0.82 ± 0.04 mm^{-1} in *ob/+* mice ($P < 10^{-4}$), whereas the mean islet area (Fig.

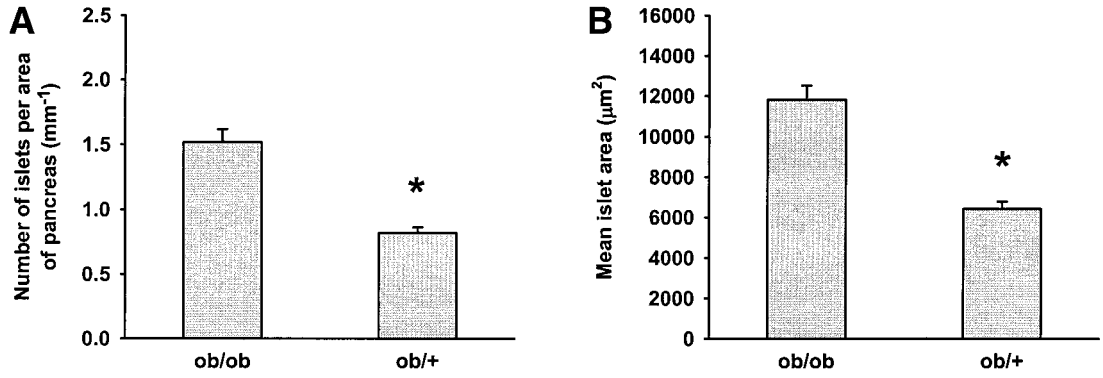

FIG. 5. The data for the number of islets per area of pancreas and the mean islet area in sections. Data are presented as group means, with the error bars representing the SE. *A*: Number of islets per area. *B*: Mean islet area. *Statistically significant difference between the *ob/ob* group and the *ob/+* group. The actual *P* values are given in the text.

5*B*) was $1.18 \pm 0.07 \times 10^4$ μm^2 in *ob/ob* mice vs. $0.64 \pm 0.04 \times 10^4$ μm^2 in *ob/+* mice ($P < 10^{-5}$). Had the number of islets per area of pancreas and the mean islet area been interpreted as reliable parameters of the total number of islets and mean islet volume, respectively, then the (erroneous) conclusion would have been that islet hyperplasia and islet hypertrophy contributed approximately equally to the increased total islet volume because the number of islets per area of pancreas and the mean islet area increased by approximately the same factor (1.85 for the number of islets per area of pancreas and 1.84 for the mean islet area) in *ob/ob* mice compared with *ob/+* mice.

DISCUSSION

Based on stereological methods such as fractionator sampling and disector counting (methods that rely on principles of the recent stereology based on validated mathematical and statistical principles), we found a marked increase in the total volume of the islets in *ob/ob* mice compared with *ob/+* mice, an increase that was entirely caused by islet hypertrophy, whereas the total number of islets was equal in the two groups with a reasonably narrow confidence interval for the difference of means.

With the definition of an islet as a group of endocrine cells containing at least three visible nuclei, we found the H&E staining to be preferable because *1*) it enables an unambiguous identification of the islets; *2*) it is easily reproducible, giving the same staining intensity in all sections; and *3*) the appearance of the islet cells by H&E staining is the same in diabetic and nondiabetic animals. An alternative would be to use immunochemically stained sections, i.e., using a cocktail of primary antibodies directed toward the islet hormones. Compared with H&E staining, this would, in theory, enable the definition of an islet to be set at only one or more endocrine cell. On the other hand, a disadvantage of immunochemical staining is that the β-cells, the most frequent cell type in the islets, gradually degranulate as the diabetic condition progresses, leaving this approach sensitive to the, at least theoretical, possibility that single or small clusters of β-cells could escape sampling in the pancreata from the *ob/ob* mice. So, although both immunochemical and physicochemical staining have their advantages and disadvantages, we considered the latter to be the most reliable for the methods applied when comparing diabetic and nondiabetic individuals.

We found a highly significant increase in the number of islet profiles per area of pancreas in *ob/ob* mice in this investigation. This result is, however, not in conflict with the finding of an unchanged total number of islets, and it is actually what is to be expected because the mean height of the islets (h in Eq. 9) increases with an increasing mean islet volume. A conflict arises only if the increased number of islet profiles per area of pancreas leads to the conclusion that the total number of islets is increased, which, as described, would be an unjustified conclusion. We found the mean islet area in *ob/ob* mice to be increased by a factor of 1.8, whereas the mean islet volume increased by a factor of 3.6. Thus, had the conclusion of an increased mean islet volume been based on measuring the mean islet profile area, this conclusion would be right in the sense that the mean islet volume did in fact increase, although the impression of the magnitude of the increase would be wrong.

It has been stated in the literature that both the number and size of islets are increased in *ob/ob* mice (3,4), with references given to earlier publications of authors such as Bleisch et al. (12), Hellman et al. (5), and Gepts et al. (6). Bleisch et al. (12) used the number of islets per area of pancreas and the mean islet area to draw the conclusion that the islets in *ob/ob* mice were more numerous as well as larger when compared with normal Swiss mice. A critical discussion of these methods is given above. Hellman et al. (5) and Gepts et al. (6) both used a method described by Wicksell (13,14) that relies on the assumption of either a spherical or ellipsoidal 3-D shape of the islets. At the time when these studies were carried out, this method was state of the art, but it has now been superseded by recent developments in stereology. Hellman et al. (5) focused primarily on the size distribution of the islets (which makes sense because this is exactly the strength of the Wicksell-based method), and the total number of islets was not calculated. However, from the data given in the article, it is clear that a calculation of the total number of islets would have resulted in an increased number of islets in *ob/ob* mice. In principle, Gepts et al. (6) used the same method and, interestingly, found an unchanged total number of islets in *ob/ob* mice compared with nonobese littermates. However, without being able to explain these

apparently diverging results, it should be mentioned that the Wicksell-based method is a much stronger tool to describe the size distribution of objects as opposed to estimating their total number.

We previously reported a linear correlation between the volume-weighted mean islet volume and the total islet volume in rats (7), an observation that suggests that the increase in the total islet volume during physiological growth in rats older than 30 days is attributable to islet hypertrophy with no contribution from islet hyperplasia, which is exactly what we found in this study using a direct stereological measurement of the total number of islets in *ob/ob* mice. On the other hand, it is clear that new islets do develop under certain experimental conditions, such as after partial pancreatectomy, where the formation of new islets has been clearly demonstrated (15). However, the expansion of the islet mass in an intact pancreas due to an increased metabolic demand and the regeneration of the pancreas after partial pancreatectomy may not be directly comparable. The formation of new pancreatic tissue after partial pancreatectomy seems to recapitulate embryonic development (15), and that may include the expression of a number of growth factors that are not present in the intact pancreas.

An obvious question is why new islets do not form even under presumably maximal growth of the total islet volume, as occurs in *ob/ob* mice. Other anatomical structures, such as kidney glomeruli, also lack the ability of hyperplasia and instead become hypertrophic with an increased demand, probably because of the highly specific structure of the neurovascular and tubular systems necessary for appropriate function. Possibly, the architecture (i.e., the intra-islet vascular structure) of the islets is complex to a degree that it only allows new islets to be formed during the formation, growth, or regeneration of the pancreas per se, as occurs during fetal life or after partial pancreatectomy. However, we do not claim that islet neogenesis per se does not occur in mice, but rather that the number of new islets eventually generated is the same in *ob/ob* mice and *ob/+* mice, despite the pronounced difference in the total islet volume.

An increased total volume of a cell population can be due to either an increased number of cells, an increased mean cellular volume, or a combination of the two. However, given the degree of the total islet mass expansion in *ob/ob* mice in both the previous investigations and the present study, it is clear that the vast majority of the islet mass expansion is the result of hyperplasia of islet cells. If the increase in total islet volume is caused entirely by the growth of existing islets, what are the primary sources for the new cells (primarily β-cells)? An obvious answer is from cells already within the islets. This could be achieved by intra-islet β-cell mitosis, an event that definitely occurs based on the presence of mitotic figures in intra-islet β-cells (6,16) and the finding of bromodeoxyuridine (BrdU) incorporation in β-cells also under normal conditions (17), or the new cells could be derived from intra-islet stem/progenitor cells, even though the existence of such cells is still under debate. Furthermore, the importance of β-cell mitosis is supported by the observation that *ob/ob* mice have an increased ratio of BrdU-positive β-cell nuclei during the expansion of the β-cell mass (18). Duct cells have also drawn much attention as cells with the capability to differentiate into β-cells (19–21), and an increased number of pancreas duodenum homeobox-1 (PDX-1)-positive cells has been found in the ducts under conditions with expansion of the β-cell mass (22,23). Based on the findings in this study, however, it could be argued that these cells are not of primary importance for the expansion of the islet mass in *ob/ob* mice, and unlike the rate of formation of duct cells positive for PDX-1 and insulin, the death rate of these cells has not been extensively studied. A possibility that should not be ignored, though, is that β-cells formed in the ducts could migrate into existing islets because the majority of islets are directly attached to the ductal tree (24).

In conclusion, the increased total islet mass in *ob/ob* mice is caused entirely by islet hypertrophia without the formation of additional islets when compared with *ob/+* mice. This suggests that dividing intra-islet β-cells and possibly intra-islet progenitor/stem cells are the primary sources for new β-cells during adaptation to an increased metabolic demand. It further suggests that the islets of Langerhans, from an anatomical point of view, are even more unique structures than previously realized, and that neogenesis of new islets is only possible under conditions such as during pancreas development/growth or after partial pancreatectomy.

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