ABSTRACT
Diabetes is characterized by rising levels of blood glucose and is often associated with a progressive loss of insulin-producing beta cells. Recent studies have demonstrated that it is possible to regenerate new beta cells through proliferation of existing beta cells or de novo formation of beta cells. The hormone insulin has a central role in the regulation of blood glucose, and the main causes of diabetes are loss of beta cells and peripheral insulin resistance, whereby beta cells fail to meet the required insulin demand (type II diabetes). For almost a century, hormone replacement therapy using insulin has been the preferred choice for treatment of diabetes (Joshi et al., 2007). However, in recent years, there has been an increased focus on finding drugs or therapies that can either prevent the destruction of beta cells or restore functional beta cell mass and thereby cure the disease (Ben-Othman et al., 2017; Li et al., 2017; Zhou et al., 2008). Consequently, the development of novel tools and models that can reliably detect changes in beta cell mass and health is needed.

RESULTS
Pipeline for 3D imaging of beta cell volume and proliferation in the entire pancreata
Fast volumetric analysis of changes in islet morphology and molecular characteristics in an entire pancreas would enable more efficient preclinical pharmacological research. Here, we set out to establish a light-sheet fluorescence microscopy 3D imaging platform with sufficient sensitivity to detect changes in beta cell volume in a mouse model of diabetes, focusing first on type II diabetes. For this purpose, mice were treated for 2 weeks with either vehicle or the insulin receptor antagonist S961 (Fig. 1A). During the in vivo phase, blood glucose and plasma insulin levels were measured at day 6 and day 13, and an oral glucose tolerance test (OGTT) was performed at day 13. At day 14, the mice were killed, and the pancreata were removed and immunolabeled with antibodies.
against insulin and Ki67 (also known as Mki67) using a modified version of iDISCO (Renier et al., 2014). Prior to clearing, the whole pancreas was embedded in low-melting-point agarose using the chamber of a 10 ml syringe, from which the tip had been removed, as a mold. The resulting agarose block that was cleared in dibenzyl ether had a diameter of \(\sim 12\) mm, which allows the entire pancreas to be scanned in one tile. This minimized overall scanning time and enabled us to maintain the same imaging settings for all samples (i.e. position in laser lines), but, as a result, the morphological features, such as head and tail, could no longer be distinguished from each other. The scanned images were processed for image analysis using the insulin channel to segment individual islets (Movie 1). This segmentation was subsequently used to quantify the total beta cell volume, the total number of insulin-positive islets and the total number of Ki67-positive beta cells (Fig. 1B; Movie 2).

**S961 treatment leads to impaired glucose handling**

The insulin receptor antagonist S961 is a single-chain peptide of 43 amino acids that binds with high affinity to the insulin receptor, but without activating it (Schäffer et al., 2008). Blocking of insulin receptor signaling using S961 has previously been shown to rapidly induce proliferation of existing beta cells (Dumayne et al., 2020; Jiao et al., 2014; Yi et al., 2013). Throughout the study, all mice had free access to high-calorie food, and the two groups consumed the same amount of food (Fig. 2A). Owing to the surgery required for implantation of the minipumps, a small decrease in body weight was observed in both groups. However, the weight loss was more pronounced in the S961 group (Fig. 2B), corresponding to the catabolic phenotype expected from impaired insulin signaling. After 13 days of treatment, the mice were subjected to an OGTT (Fig. 2C). At all points measured, the S961-treated mice displayed significantly higher blood glucose levels, and the return to baseline was delayed. Similarly, both plasma insulin levels and fasting blood glucose were markedly increased in the S961-treated group (8.15 mmol/l and 17.1 mmol/l in the vehicle and S961-treated group, respectively; Fig. 2D,E). At termination, the whole pancreas was removed and weighed. No difference in pancreas weight was observed between the two groups (Fig. 2F). Taken together, these observations indicate that S961 effectively blocked insulin signaling, leading to a diabetic phenotype.

**Increased beta cell volume in S961-treated mice**

In order to determine the effect of the metabolic changes on beta cell volume, the pancreata from all mice were immunolabeled with antibodies against insulin and the cell proliferation marker Ki67 using a modified iDISCO protocol (Renier et al., 2014). The insulin-segmented signal (Fig. 3A) also allowed quantification of the total number of insulin-positive islets and beta cell volume in each pancreas. A low-size cut-off of \(25 \times 1000 \mu m^3\) for islets was implemented to avoid false-positive signal that may result, for example, from the non-specific presence of fluorophore in the tissue (from fluorophore-conjugated secondary antibody). The mean number of insulin-positive islets was \(7962 \pm 241\) in the vehicle mice and \(9631 \pm 346\) in the S961-treated mice, corresponding to a 21% increase in the S961 group (Fig. 3B). Similarly, the mean beta cell volume in the pancreas was...
2.95±0.15 mm³ in the vehicle group and 4.23±0.28 mm³ in the S961 group, corresponding to a 43% increase in the S961 group (Fig. 3C,D). Hence, S961 treatment resulted in a significant (P≤0.001) increase in both islet count and volume.

**Islets of all sizes are affected by S961 treatment**

Because there is considerable interest in defining the heterogeneity of islets and the response of different size islets to diabetes and drug treatment (Aizawa et al., 2001; Baetens et al., 1979; Lehmann et al., 2007; Saito et al., 1978), we allocated the segmented islets into four size bins: small (yellow; 25-170×1000 µm³), medium (cyan; 170-1100×1000 µm³), large (magenta; 1100-7500×1000 µm³) and very large (red; 7500-50000×1000 µm³), and charted the location of these categories in all pancreata (Fig. 3C; Fig. S1). When looking at the accumulated beta cell volume as a function of individual insulin-positive islet size, it is evident that there was a continuous increase in the total beta cell volume following S961 treatment across the islet size categories (Fig. 4A,B).

To characterize the changes in more detail, we looked at the islet size categories separately (Fig. 4C). In the small insulin-positive islet category, our analysis counted 3318±141 islets in the vehicle group and 3614±102 islets in the S961 group, which corresponds to an 8.9% increase in the S961 group. For the medium islet category, the numbers were 2300±99 in the vehicle group and 3458±299 in the S961 group, representing a 50.3% increase in the S961 group (P≤0.05). In the large islet category, there were 456±28 islets in the vehicle group and 621±36 in the S961 group, corresponding to a 36.1% increase in the S961 group. In the very large islet category, there were 45±5 islets in the vehicle group and 68±9 in the S961 group, corresponding to a 51.1% increase in the S961 group. Statistical analysis of the islet count data revealed significant main effects of both treatment [F(1,12)=18.37, P=0.0011] and size category [F(3,36)=356.93, P<0.001], and their two-way interaction was also found to be significant [F(3,36)=8.16, P=0.0045].

Next, we examined the effect of S961 on beta cell volume in the different-sized bins (Fig. 4D). In the small islet category, the beta cell volume was 0.26±0.01 mm³ in the vehicle group and 0.3±0.01 mm³ in the S961 group, representing a 15.3% increase in the S961 group. For the medium-sized islets, we detected a 50.5% increase in beta cell volume (P≤0.05), from 0.93±0.05 mm³ in the vehicle group to 1.4±0.1 mm³ in the S961 group. For the large islets, the beta cell volume was 1.19±0.07 mm³ in the vehicle group and 1.6±0.09 mm³ in the S961 group, corresponding to a 34.4% increase in the S961 group. Finally, for the very large islets, beta cell volume was 0.55±0.07 mm³ in the vehicle group and 0.9±0.15 mm³ in the S961 group, corresponding to a 97.8% increase in the S961 group. For the islet volume data, statistical analysis revealed significant main effects of both treatment [F(1,12)=15.87, P=0.0018] and size category [F(3,36)=86.28, P<0.001], but no significant two-way interaction was found [F(3,36)=3.39, P=0.0513]. As the islet count data and the beta cell volume data are correlated measures, pairwise post hoc tests on the treatment effect within each size category were carried out in a multivariate manner using Hotelling’s T-squared test. The pairwise tests showed significant treatment effect for small (P=0.0233), medium (P=0.0094) and large islets (P=0.0121), but not for very large islets (P=0.1694).

**Proliferation of beta cells is the most likely explanation for increased beta cell volume in response to S961 treatment**

By staining pancreata for Ki67, we set out to quantify the number of proliferating beta cells. Light-sheet microscopy enabled the identification of individual proliferating cells within 3D-imaged...
pancreata (Fig. 5A,B). Using insulin staining to segment the beta cell volume, we were able to quantify Ki67-positive nuclei within this volume. The analysis illustrates a significant increase in the total number of Ki67-positive islets and in the total number of proliferating cells within the beta cell volume across pancreata in the S961 group in comparison to the vehicle group (Fig. 5C,D). Quantification of Ki67 signal by islet size categories demonstrates that there was an increase in proliferation among islets of all analyzed sizes, albeit with variation between samples (Fig. 5E). In statistical analysis of the number of Ki67-positive islets, significant effects were found for both treatment \( [F(1,12)=10.92, P=0.0063] \) and size category \( [F(3,36)=180.62, P<0.001] \). No significant two-way interaction was observed \( [F(3,36)=1.03, P=0.3590] \).

Unpaired Student’s t-test were performed on the treatment effect within each size category, which indicated significance within all size categories: small \( (P=0.0129) \), medium \( (P=0.0072) \), large \( (P=0.0189) \) and very large \( (P=0.0051) \).

**Identification of early signs of type I diabetes in mouse using light-sheet imaging**

Female NOD mice develop hyperglycemia at ~14 weeks of age (males a few weeks later) (Mathews et al., 2015). The onset is variable, and, at 5 weeks, immune cell infiltrates can already be detected (DiLorenzo et al., 1998). We reasoned that 3D light-sheet imaging can capture the inflammatory changes in pre-diabetic NOD mice and thus analyzed pancreata from 14-week-old female mice. At the time of termination, blood glucose was normal (data not shown). The dissected pancreata were stained for insulin and the general leukocyte marker CD45 (also known as PTPRC) (Fig. 6A; Movie 3). Light-sheet microscopy enabled the visualization of leukocytes across pancreata, although in highly inflamed areas individual cells could not be distinguished as CD45 antigen protein tyrosine phosphatase receptor type C is a membrane protein (Fig. 6A,B). Using the islet segmentation platform as described above, we identified insulin-labeled islets, classified these into size categories and quantified CD45 staining within these as a fraction of total islet volume (Fig. 6C). A 5% volume threshold (CD45 signal from total islet volume) was implemented to define an inflamed islet. We observed that 42.1±11.7% of islets in the small islet category, 68.8±13.7% of islets in the medium islet category, 85±6.4% of islets in the large islet category and 100% of the islets in the very large islet category were positive for CD45 (Fig. 6D). These data demonstrate extensive islet infiltration in NOD mice at the onset of diabetes, whereby a large majority of medium- to large-sized islets are already inflamed.

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**Fig. 3. Increased beta cell volume and number of insulin-positive islets following S961 treatment.** (A) Using the insulin signal, each islet was assigned a unique ID and a volume, making it possible to calculate the total number of insulin-positive islets and the total beta cell volume. (B) Total number of insulin-positive islets in vehicle- and S961-treated mice. (C) The same pancreas as shown in A, color coded for size distribution. The insulin-positive islets were allocated into four bins: small (yellow; 25-170×1000 µm³), medium (cyan; 170-1100×1000 µm³), large (magenta; 1100-7500×1000 µm³) and very large (red; 7500-50000×1000 µm³). (D) Total mean beta cell volume in the study groups. Individual data points are indicated. For statistical analysis, we used unpaired Student’s t-test. ***P<0.001, S961 compared to vehicle. Error bars represent s.e.m. Scale bars: 500 µm.
DISCUSSION

The aim of this study was to demonstrate a method for accurate measurement of islet beta cell volume, proliferation and inflammation in mice using light-sheet microscopy and automated 3D image analysis. In particular, 3D imaging of entire pancreata enabled us to reveal the changes in different islet size categories. Using the herein developed methods, we show that, in a mouse model of S961-induced hyperglycemia and impaired insulin sensitivity, there is an overall increase in beta cell volume. In the NOD mouse model of type I diabetes, we show that, prior to the onset of hyperglycemia, more than 80% of large islets are already infiltrated by leukocytes.

A particular strength of 3D light-sheet imaging is the capacity to carry out volumetric analysis of islets in different size categories and to characterize various parameters within these. Heterogeneity of islets and functional differences in small and large islets are well established (Aizawa et al., 2001; Baetens et al., 1979; Huang et al., 2011; Roscioni et al., 2016). It is, for example, conceivable that different sources of beta cell regeneration may impact islet size distribution differently, and accurate analysis of the process is needed for preclinical drug efficacy studies. Neogenesis or transdifferentiation may increase the number and volume of small islets more than those of large islets, while general beta cell proliferation may result in a more uniform response across size categories.

To study the response to acquired impairment in insulin sensitivity, we used the insulin receptor antagonist S961. Chronic dosing in male high-fat-diet-induced obese mice resulted in increased levels of insulin and blood glucose, indicating that the S961 dose chosen was sufficient to drive a sustained physiological response. Our 3D image analysis demonstrated an overall increase in beta cell volume and in

Fig. 4. S961 treatment response in different islet size categories: (A) Size distribution curve of islets. Size categories as indicated in the Fig. 3 legend. (B) Relative contribution of islets in different size categories to the total islet volume. Islets across all size categories contribute to the overall increase in total beta cell volume. (C) Quantification of the number of small (yellow), medium (cyan), large (magenta) and very large (red) insulin-positive islets in vehicle- and S961-treated mice. In all four bins, there is an increase in the number of insulin-positive islets following S961 treatment. (D) Quantification of the beta cell volume in the small (yellow), medium (cyan), large (magenta) and very large (red) islets in vehicle- and S961-treated mice. Individual data points are indicated. In C and D, both sets of data were separately investigated using 2×4 mixed ANOVA, and in follow-up tests on the treatment effect within each size category, the two measures were investigated in a multivariate manner (owing to correlation) by Hotelling’s T-squared test. *P<0.05, **P<0.01; S961 compared to vehicle. Error bars represent s.e.m.
islet count. Analysis of the response in islet size categories showed an increase in both the number and volume of small-, medium- and large-sized islets in the S961-treated group. The number of islets and their mean volume is well known to depend on disease phase in patients with type II diabetes. An early compensatory hypertrophy of islets is later followed by beta cell loss (Chen et al., 2017). In support of this, the presented Ki67 analysis showed an overall increased labeling in the S961 group, suggesting higher beta cell proliferation. This result corresponds with previous studies showing that beta cell proliferation is the main factor driving the expansion of beta cell volume in response to S961 dosing (Dumayne et al., 2020; Jiao et al., 2014; Yi et al., 2013). Similarly, increased islet number in S961-treated mice has also been observed by other groups (González-Mariscal et al., 2018; Okamoto et al., 2017). However, these results do not rule out alternative mechanisms resulting in increased beta cell count and volume. These include trans-differentiation of other cell types into beta cells and islet neogenesis. Other islet cell types (Ben-Othman et al., 2017; Cigliola et al., 2018; Li et al., 2017; Zhou et al., 2008) and ductal epithelium (Bonner-Weir et al., 2012) may contribute to insulin-producing cells. More detailed analysis of different cell types in early phases of S961 treatment could provide clues into biological processes in early type II diabetes.

Type I diabetes results from autoimmune destruction of beta cells. The NOD mouse model has provided valuable insights into the disease progression, but direct translation to new therapeutics has been challenging (Pearson et al., 2016; Reed and Herold, 2015; Sandor et al., 2019). High variability, both in NOD mice and in patients, and still inadequately understood mechanisms of the immune response have hindered pharmacological advances. Here, we established a light-sheet imaging platform to characterize immune cell infiltration in islets in the NOD model. We show that, before any changes in blood glucose can be detected, 80% of large islets and 40% of small islets are infiltrated by CD45+ immune cells. Previous intravital imaging studies, using fluorescently labeled immune cell subsets have established the early onset of the disease at 3-5 weeks of age, whereby stochastic homing and infiltration of islets occur at first by individual infiltration of autoreactive T-cells (Coppieters et al., 2012; Lindsay et al., 2015; Mohan et al., 2017). This early infiltration occurs significantly prior to the development of hyperglycemia at 14 weeks (Mathews et al., 2015). After the initial infiltration of the islets, they are targeted by multiple leukocyte subtypes from the islet periphery (Mohan et al., 2017). In support of this, we found accumulation of leukocytes in the islet periphery at 14 weeks. The light-sheet imaging platform demonstrated in the present study does not rely on transgenic
reporters or in vitro cell labeling and is particularly amenable to pharmacological research in type I diabetes. Additional antibodies for immune cell subsets can be included, enabling the definition of changes in the immune cell repertoire.

In conclusion, we demonstrate here a 3D imaging platform for the analysis of changes in entire pancreata in mouse models of type I and II diabetes. We show that the insulin receptor antagonist S961 leads to increased islet number and volume, likely due to proliferative response within beta cells. In the NOD mouse model of type I diabetes, we found that, prior to the onset of hyperglycemia, the vast majority of islets already show significant leukocyte infiltration. The established methodology can be adjusted to incorporate additional markers of interest (i.e. activated cell signaling pathways, leukocyte subsets, drug target receptors) for gaining better understanding of the basic biological mechanisms of type I and II diabetes and for pharmacological analysis of the efficacy of therapeutics.

MATERIALS AND METHODS

Animals
Male C57Bl/6J mice were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and maintained in standard housing conditions (12 h light/dark cycle and controlled temperature of 21-23°C). Mice had ad libitum access to tap water and 60% high-fat diet (high-fat Ssniff diet D12492; Brogaarden, Hørsholm, Denmark; 60% kcal% fat (91% lard, 9% soybean oil), 20% kcal% protein (98.5% casein, 1.5% L-cystine), 20% kcal% carbohydrate (63% Lodex-10, 37% sucrose). Groups were age matched (30 weeks). When the mice reached a body weight of ∼50 g, they were randomized into two groups according to body weight (n=7). Alzet osmotic minipumps (1002; Alzet, Cupertino, CA, USA) were implanted, containing either vehicle (PBS) or...
Sample preparation for immunohistochemistry

Animals were transcardially perfused with heparinized PBS and 40 ml of 10% neutral buffered formalin (CellPath, Newtown, UK) under Hypnorm-Dormitrium (fentanyl 788 μg/kg, fluanisone 25 mg/kg and midazolam 12.5 mg/kg, subcutaneous injection) anesthesia. Pancreata were carefully dissected and immersion fixed in 10% neutral buffered formalin overnight at room temperature on a horizontal shaker. The samples were washed 3×30 min in PBS with shaking and dehydrated at room temperature in methanol/H2O gradient to 100% methanol (20%, 40%, 60%, 80%, 100% methanol; each step 1 h). The pancreata were stored in 100% methanol (VWR International A/S, Søborg, Denmark) at 4°C until further processing.

Whole-pancreas immunohistochemistry for labeling of insulin and Ki67

For whole-pancreas immunohistochemistry, a modified version of the original iDISCO (immunolabeling-enabled three-dimensional imaging of solvent-cleared organs) protocol was used (Renier et al., 2014, 2016). Samples were washed with 100% methanol for 1 h and incubated overnight in 66% dichloromethane/33% methanol (VWR International A/S) at room temperature. Then, samples were washed twice in 100% methanol for 30 min and bleached in chilled fresh 5% H2O2 (Acros Organics, Fisher Scientific Biotech Line A/S, Sølangerup, Denmark) in methanol overnight at 4°C. Subsequently, the samples were rehydrated in methanol/PBS series (80%, 60%, 40%, 20% methanol with 0.2% Triton X-100 (Merck, Darmstadt, Germany); each step 1 h) at room temperature, washed in PBS with 0.2% Triton X-100 twice for 1 h at room temperature and in permeabilization solution [PBS with 0.2% Triton X-100, supplemented with 20% volume dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and 2.3% weight/volume glycine (Merck)] for 3 days at 37°C. Unspecific antibody binding was blocked by a 2-day incubation in blocking solution [PBS, 2% Triton X-100, 10% DMSO] 6% donkey serum (Jackson ImmunoResearch, Cambridge, UK)]. Immunohistochemistry was carried out sequentially, by first incubating the samples for 7 days at 37°C with anti-Ki67 antibody (1:200 dilution; NBI10-89717; Novus Biologicals, Centennial, CO, USA) or with anti-CD45 (1:200 dilution; 550539; BD Pharmingen, CA, USA), diluted in PTwH [PBS, 0.2% Tween 20 (Merck), 0.1% of 10 mg/ml heparin solution], 5% DMSO, 3% donkey serum, 0.2% of 10% NaNO3 (Merck). Following incubation with primary antibody, the samples were washed in PTwH for 1×10 min, 1×20 min, 1×30 min, 1×1 h, 1×2 h and 1×2 days. Subsequently, the pancreata were incubated in secondary antibody solution (PTwH, 3% donkey serum, 0.2% of 10% NaNO3) for 7 days at 37°C with Alexa Fluor 790 AffiniPure Donkey Anti-Rabbit IgG (1:1000 dilution; 711-655-152; Jackson ImmunoResearch) or Anti-Rat-Cy3 IgG (1:1000 dilution; 712-165-153; Jackson ImmunoResearch) and washed in PTwH for 1×10 min, 1×20 min, 1×30 min, 1×1 h, 1×2 h and 1×3 days. Samples were post-fixed in 10% neutral buffered formalin overnight and incubated in Alexa Fluor 647-conjugated anti-insulin antibody (1:500 dilution; 9008; Cell Signaling Technology, Danvers, MA, USA) in the above-described antibody dilution buffer. The samples were subsequently washed again in PTwH for 1×10 min, 1×20 min, 1×30 min, 1×1 h, 1×2 h and 1×3 days and embedded in 1% low-melting-point agarose (16520050; Thermo Fisher Scientific; dissolved in PBS). The agarose-embedded pancreata were dehydrated in increasing concentrations of methanol (20%, 40%, 60%, 80%, 100%; 1 h each at room temperature), followed by overnight incubation in 100% methanol. Samples were next incubated in 66% dichloromethane/33% methanol for 3 h at room temperature with shaking and in 100% dichloromethane twice for 15 min with shaking to remove traces of methanol. Finally, the samples were transferred to dibenzyl ether (Merck) and stored in closed glass vials until imaging with light-sheet fluorescence microscopy.

Light-sheet fluorescence microscopy of cleared immunolabeled pancreata

All agarose-embedded pancreata were imaged on a LaVision ultramicroscope II setup (Miltényi Biotec, Bergisch Gladbach, Germany) equipped with a Zyla 4.2-PCL10 sCMOS camera (Andor Technology, Belfast, UK), SuperK EXTREME supercontinuum white-light laser EXR-15 (NKT Photonics, Birkerød, Denmark) and MV PLAPO 2XC (Olympus, Tokyo, Japan) objective lens. The samples were attached to the sample holder with neutral silicone and imaged in a chamber filled with dibenzyl ether. Version 7 of the Inspektor microscope controller software was used. Images were acquired at 0.63× magnification (1.2× total magnification) with an exposure time of 266 ms for insulin and 1 s for Ki67 in a z-stack at 10-µm intervals. Acquired volumes (16-bit tiff) had an in-plane resolution of 4.8 μm and z-resolution of 3.78 μm (NA=0.156). High numerical aperture is needed to capture individual Ki67+ cell nuclei; however, this results in uneven light-sheet thickness. To alleviate the effect of this, horizontal focusing was captured in nine planes with contrast-based blending of the images.

Image processing for insulin and CD45 segmentation

U-Net network architecture (Ronneberger et al., 2015) was used to create a 2D U-Net with four repeated layers for encoding and four repeated layers for decoding, implemented in Python utilizing the Keras machine learning library (https://github.com/keras-team/keras). The U-Net input was a single intensity channel and the output was a single label image. Raw images were downsampled by a factor of 2 to a size of 1024×1024 pixels. Annotations were performed manually on a total of 154 image tiles with a size of 512×512 pixels. Intensities of the training images were rescaled between 0 and 1; 75% of the data were used for training, 20% for validation and 5% for testing. Data augmentation, in the form of skews, rotations, flips, zoom and random distortions, was applied during training with probability of 30% for each operation. Training was performed for 350 epochs and the model achieved a dice coefficient of 0.79 on the validation set. The trained model was afterwards used to segment full-size 2048×2048 pixel images. For an example of the segmentation see Fig. S2.

Quantification of Ki67-positive cells

Background subtraction through morphological opening using a disk element was performed slice-by-slice on raw Ki67 intensity images. To identify Ki67-positive cell candidates, local intensity peaks were located by moving a filter cube [5×5×3 (x,y,z) voxels] over the image volume. The coordinates of detected local intensity peak candidates were used as seeds in a watershed segmentation with a background intensity cut-off at 50, and the resulting segmentations were filtered by removing cell segmentation regions smaller than 4 voxels and bigger than 100 voxels.

Statistics

Pairwise treatment effects were investigated using unpaired Student’s t-tests or Hotelling’s T-squared test. Additionally, in multiple cases, the treatment effect was investigated specifically for different islet size categories. This required binning the data into four different size categories. As the binnings were splits of the full data set, this introduced a correlated factor to the analysis. Thereby, data were analyzed as a two-way 2×4 (two treatments, four size categories) mixed ANOVA, with the treatment as a between-subjects factor and the size category as a within-subjects factor. For all mixed ANOVAs, the Greenhouse–Geisser correction was used for P-values related to the within-subjects factor, if Mauchly’s test of sphericity indicated that the assumption of sphericity was violated. All statistics were carried out
using R (https://www.r-project.org/), and statistical results are commonly presented alongside mean±s.e.m.

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Competing interests


Author contributions


Data availability

The source code used for generating the insulin segmentation data is accessible at https://github.com/Gubra-ApS.

Financial support

Supplementary information

Supplementary information available online at https://dmdm.biologists.org/lookup/doi/10.1242/dmd.045351

References


**Figure S1. Overview of all pancreata in the two groups:** For size distribution the insulin positive islets were allocated into four bins, small (yellow; 25-170 x1000 µm³), medium (cyan; 170-1100 x1000µm³), Large; (magenta; 1100-7500 x1000µm³) and very large (red; 7500-50000 x1000µm³).
Figure S2. Computational identification of inflamed islets: Computational identification of CD45 signal (in greyscale, note that large black areas represent lymph nodes), insulin signal in the middle panel and on the right, identification of insulin positive islets in 3D imaged pancreas with CD45 signal.
Movie 1: Segmentation of the insulin signal: the segmentation of individual islets based on insulin signal (cyan).

Movie 2: Mouse pancreas immunolabelled with insulin and Ki67: A whole mouse pancreas from a S961 dosed mouse labelled with antibodies against insulin (cyan) and Ki67 (glow scale).

Movie 3: Double labelling of NOD pancreas: Movie showing the infiltration of pancreatic islets in a NOD mouse. The pancreas was stained for insulin (cyan) and CD45 (glow scale).