Electronic supplementary material

Methods

Subjects The study was performed on pancreatic samples obtained at autopsy (within 12 h of death) of the individuals in whom we previously measured the beta cell mass [7]. All the 52 non-diabetic subjects could be studied again, but adequate samples were available from only 50 of the 57 type 2 diabetic subjects. Omission of these seven type 2 diabetic subjects has no significant impact on the comparisons between non-diabetic and type 2 diabetic groups and on the conclusions of our previous study. For instance, beta cell mass averaged 567±248 mg in these 50 type 2 diabetic subjects as compared with 573±259 mg in the whole group of 57 subjects [7].

Pancreas processing and microscopic examination All available samples were fixed in Bouin–Allen solution and embedded in paraffin. Sections of 5 µm were cut and first immunostained for insulin using the monoclonal anti-insulin antibody 9569-100, 1/500 (Abcam, Cambridge, UK) revealed by the UltraView Universal Diaminobenzidine detection kit from Ventana (Tucson, AZ, USA). They were then stained for glucagon using the antiserum NCL-GLUCp, 1/400 (Novocastra, Newcastle upon Tyne, UK) revealed by the UltraView Universal Alkaline Phosphatase Red detection kit from Ventana. Finally, the sections were counterstained by haematoxylin to facilitate recognition of tissue structure.

Stained pancreatic sections were analysed with a Zeiss Axioplan microscope (Oberkochen, Germany), using a 40× plan neofluar objective and a final magnification of 400×. For all subjects, one section from the body of the pancreas was analysed entirely and, for subsets of 30 non-diabetic and 30 type 2 diabetic subjects, one section from the tail was also analysed. The ratio of alpha cell to beta cell areas was determined by a point counting method using a 100 point lattice [20]. In practice, the number of points corresponding to alpha cells (in red) and beta cells (in brown) were counted for each islet in all fields. Small structures of a few cells were included in the analysis, but single cells were not counted. The total number of points (alpha cells + beta cells) so counted

averaged 1,794 (696–4,064) in non-diabetic subjects and 1,451 (698–3,266) in type 2 diabetic subjects.

Analysis of the results In our previous study, both the weight of the pancreas and the beta cell volume density (Vv) were measured in all samples, permitting calculation of the beta cell mass [7]. This calculation took into account the higher Vv measured in the tail than in the body of the pancreas, and was corrected for the 50% lower Vv of beta cells known to exist in the lobe rich in pancreatic polypeptide cells in the posterior part of the head [15, 17]. The current measurements of the ratio of alpha:beta cell areas were used to calculate the alpha cell mass in the following way. Because the ratio of alpha:beta cell areas was only minimally and inconsistently higher in the tail than in the body (see Results), calculation of the alpha cell mass using these two values separately, their mean or the value in the body only yielded similar results (average difference of 2–3%). We therefore used mean ratio for body and tail when two values were available and ratio for body in the other cases. A correction was applied for the virtual absence of alpha cells in the lobe rich in pancreatic polypeptide cells [15, 17], which contains about 7.5% of the total beta cell mass [7]. We therefore multiplied the ratio of alpha:beta cell areas by 92.5% of the known beta cell mass to obtain the alpha cell mass in each subject.

In 24 subjects from each group we also compared the ratio of alpha:beta cell areas in islet profiles of different sizes. Five classes of islet size were defined according to the total number of points (alpha + beta) counted in each profile: 1–5, 6–10, 11–15, 16–20 and >20. The smallest category corresponds to an area between 625 and 3,125 μm^2 (6–27 cells) and the largest one to >12,500 μm^2 (>110 cells). However, these are minimum estimates because the examined islet profiles also contained structures (vessels, non-stained cells) that were not counted.

Presentation of results Most figures show scatter plots of individual values. As all sets of data passed the Kolmogorov–Smirnov test of normality, values for non-diabetic and type 2 diabetic groups are presented as means ± SD, and the differences assessed by Student's *t* test. Comparisons using the non-parametric Mann–Whitney *U* test yielded similar levels of significance.