The handle http://hdl.handle.net/1887/32608 holds various files of this Leiden University dissertation.

**Author:** Ellenbroek, Johanne Hendrike (Rianne)
**Title:** Pancreatic β- and α-cell adaptation in response to metabolic changes
**Issue Date:** 2015-25-03
Chapter 4

Topologically heterogeneous $\beta$- and $\alpha$-cell adaptation with maintenance of $\alpha$- to $\beta$-cell ratio in obesity

Johanne H. Ellenbroek$^1$, Hendrica A. M. Töns$^1$, Maaie A. Hanegraaf$^1$, Ton J. Rabelink$^1$, Marten A. Engelse$^1$, Françoise Carlotti$^1$, Eelco J. P. de Koning$^{1,2,3}$

$^1$Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands; $^2$Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands; and $^3$Hubrecht Institute, Utrecht, The Netherlands

Submitted
Abstract

Background/Objectives
In order to maintain glucose homeostasis the number and/or function of insulin-producing pancreatic β-cells can change. It is unknown whether β-cell adaptation is homogeneous throughout the pancreas of human subjects. Hyperglucagonemia is present in type 2 diabetes, but data are lacking whether the glucagon-producing α-cells adapt to changes in weight. In this study we examined β- and α-cell mass of non-diabetic obese and lean human subjects throughout different regions of the pancreas.

Subjects/Methods
Pancreatic tissue of the head-, body- and tail-region of the pancreas was examined from 15 obese organ donors with a Body Mass Index (BMI) ≥ 27 kg/m² and age-matched lean organ donors with a BMI ≤ 25 kg/m². β- or α-cells were identified by immunostaining for insulin and glucagon, respectively.

Results
In obese subjects β- and α-cell mass were proportionally increased compared to lean subjects (β-cell mass 2.4±0.3 g (obese) vs. 1.6±0.2 g (lean), p<0.05; α-cell mass 1.2±0.2 g (obese) vs. 0.8±0.1 g (lean), p<0.05), thereby maintaining the α- to β-cell ratio. While the fractional β- and α-cell area were the highest in the pancreatic tail, the homeostatic adaptation to obesity occurred preferentially in the head of the pancreas.

Conclusions
In obese subjects β- and α-cell mass are increased and this adaptation is topologically heterogeneous. As data so far have been derived from studying β- and α-cell mass in tissues from the tail-region of the pancreas, the homeostatic adaptive capacity of humans to obesity has previously been underestimated.
Introduction

Both type 1 and type 2 diabetes are characterized by the loss of functional pancreatic β-cell mass (1). For these patients therapies are needed that restore, maintain or prevent loss of functional β-cells. Therefore, it is critical to understand how the β-cell mass is regulated. In order to maintain glucose homeostasis the number and/or function of insulin-producing pancreatic β-cells can change. In human subjects it is well established that obesity and pregnancy are associated with an increased β-cell mass (2–9). However, several of these studies rely on tissue sampling from the pancreatic tail-region of the pancreas only (4, 5, 7, 9). We have recently shown that high-fat diet induced insulin resistance leads to topologically heterogeneous β-cell adaptation in mice, that is most prominent in the splenic region of the pancreas (10). In human subjects it is not known whether β-cell adaptation is homogeneous throughout the pancreas of human subjects.

Type 2 diabetes is also characterized by hyperglucagonemia and associated with an increased α-to β-cell ratio (11). In two separate studies we have recently shown that in addition to the β-cell mass, the glucagon-producing α-cell mass can also be modulated by dietary changes in mice (12, 13). It is unknown whether obesity modulates the α-cell mass and if so, how this affects the α- to β-cell ratio. In this study we examine the α- and β-cell mass in obese subjects and compare these to the findings in lean subjects as an indication of β- and α-cell mass adaptation to obesity.

Materials and methods

Subjects

Human pancreata were procured through a multiorgan donor program. Pancreatic tissue was used in our study if the tissue could not be used for clinical islet transplantation, according to national laws, and if research consent was present. Pancreatic tissue of the head-, body- and tail-region of the pancreas was examined from obese organ donors with a Body Mass Index (BMI) ≥ 27 kg/m² (n=15) and age-matched lean organ donors with a BMI ≤ 25 kg/m² (n=15). None of the organ donors had a clinical history of diabetes. Characteristics of the studied subjects are given in supplementary table 1.

Immunohistochemistry

Pancreas samples were fixed overnight in 4% formaldehyde (Klinipath, Duiven, The Netherlands), embedded in paraffin and sliced into 4 μm sections. Pancreatic polypeptide positive cells were identified in the head-regions of the pancreas using rabbit anti-PP IgG (Millipore, Billerica, MA, USA) for 30 min followed by horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h. Sections were developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB) and counterstained with haematoxylin. If microscopic examination revealed a PP-cell rich area the head-region of this pancreas was excluded from the analysis.
For identification of β-cells pancreas sections were immunostained with rabbit anti-insulin IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or guinea pig anti-insulin IgG (Millipore) for 1 h followed by HRP- or alkaline phosphatase-conjugated secondary antibodies for 1 h. α-Cells were identified by immunostaining using rabbit anti-glucagon IgG (Vector Laboratories, Burlingame, CA, USA) for 1 h followed by a HRP-conjugated secondary antibody for 1 h. To identify proliferating β- or alpha-cells, sections were double stained with mouse anti-Ki67 (Becton Dickinson, Franklin Lakes, NJ) and primary antibodies against insulin or glucagon, respectively, overnight at 4°C after heat-induced antigen retrieval in 0.01 M citrate buffer followed by HRP- and AP-conjugated secondary antibodies. Sections were developed with DAB or liquid permanent red (LPR, Dako, Denmark) and counterstained with haematoxylin. Stained sections were digitally imaged (Panoramic MIDI, 3DHISTECH, Budapest, Hungary).

**Morphometry**

For determining the fractional β-cell area, the percentage of insulin-DAB stained area out of total pancreas area stained with hematoxylin was determined using an image analysis program (Stacks 2.1, LUMC, (10)), excluding large blood vessels, larger ducts, adipose tissue and lymph nodes. The surface area of each insulin-positive cell cluster was measured and used to calculate the average β-cell cluster area per pancreatic region. Islet density was determined by dividing the number of β-cell clusters by the (regional) area that was analyzed. For measurements in the entire organ, the average of the three regions was calculated. The pancreas weight was estimated by use of an equation based on the population data from our own institute (supplemental figure 1, formula: pancreas weight = 2.31 x BMI + 47.7). β-Cell mass was determined by the β-cell area multiplied by the estimated pancreas weight. The fractional α-cell area was determined by calculating the ratio of α-cell area to β-cell area of 20 randomly selected islets per pancreatic region, using Stacks 2.1. α-Cell mass was calculated by multiplying the α- to β-cell ratio by the β-cell mass.

**Statistical analysis**

Data are presented as means ± SE. Statistical calculations were carried out using GraphPad Prism 5 (GraphPad Software, San Diego, CA). The statistical significance of differences was determined by Mann-Whitney test, or two-way ANOVA, followed by Bonferroni’s multiple-comparisons test, as indicated. Correlations were tested using Spearman rank analysis. P < 0.05 was considered statistically significant.
Results

Increased β- and α-cell mass in obese subjects

The average age of obese and lean subjects was similar (53.2±3.3 years (lean) vs. 52.4±3.0 years (obese), p=0.86; table S1). The average body mass index (BMI) was 30.1±0.6 kg/m² for obese and 22.5±0.3 kg/m² for lean subjects. The β-cell mass was determined by analysing 22.1±1.4 mm² pancreatic tissue per donor. The β-cell mass was ~1.5x higher in obese compared to lean subjects (Fig. 1A-C). No significant difference in β-cell cluster area was observed (Fig. 1D) whereas a tendency (p = 0.06) for a higher islet density was present in obese subjects (Fig. 1E). A positive correlation was found between β-cell mass and BMI (Fig. 1F, r=0.32, p<0.05).

α-Cell mass was found to be 50% increased in obese subjects (Fig. 1A, B and G). A similar α- to β-cell ratio was observed in lean and obese subjects (Fig. 1H) resulting in a strong positive correlation between β- and α-cell mass (Fig. 1I). No correlation was found between age and β-cell mass (r=0.19, p=0.16) or α-cell mass (r=-0.28, p=0.08) but a negative correlation was found between age and the α- to β-cell ratio (Fig. 2, r=-0.55, p<0.01).
Fig. 1. β- And α-cell mass in lean and obese subjects. A. Representative picture of β-cells (red) and α-cells (brown) in islets of a lean subject. Scale bar = 100 μm. B. Representative picture of β-cells (red) and α-cells (brown) in islets of an obese subject. Scale bar = 100 μm. C. β-Cell mass (n = 15). D. β-Cell cluster area (n = 30). E. Islet density (n = 30). F. Correlation between β-cell mass and BMI (n = 30). G. α-Cell mass (n = 12-14). H. α- to β-cell ratio (n = 12-14). I. Correlation between β- and α-cell mass (n = 26). *p<0.05 by Mann-Whitney U test.
Topologically heterogeneous β- and α-cell adaptation in obese humans

**Fig. 2.** Correlation between age and α- to β-cell ratio (n = 26).

β- and α-cell area are topologically heterogeneous throughout the pancreas

To assess the distribution of the β- and α-cells throughout the pancreas, the fractional β- or α-cell area, islet density, islet size and α- to β-cell ratio were determined by pancreatic region (i.e. head, body and tail). In the tail-region of the pancreas both β- and α-cell area were significantly higher compared to the head and body region in lean subjects (Fig. 3A and D). Islet density was similar throughout the pancreas in lean individuals (Fig. 3B) whereas β-cell cluster area was significantly higher in the tail-region of the pancreas compared to the head-region in lean donors (Fig. 3C). The α- to β-cell ratio was similar throughout the pancreas (Fig. 3E).

**Fig 3.** β- And α-cell area in lean and obese subjects. A. Fractional β-cell area by pancreatic region (n = 10-15 per region). B. Islet density by pancreatic region (n = 10-15). C. Mean β-cell cluster area by pancreatic region (n = 10-15). D. Fractional α-cell area by pancreatic region (n = 10-14). E. α- to β-cell ratio by pancreatic region (n = 10-14). *p<0.05 and **p<0.01 by two-way ANOVA followed by Bonferroni's multiple comparisons test, #p<0.05 and ##p<0.01 by Mann-Whitney U test.
β- and α-cell adaptation are topologically heterogeneous in obese human subjects

In obese subjects, islet density was increased in the tail-region of the pancreas (Fig. 3B). When comparing regional islet cell areas between lean and obese individuals, the β-cell area was found to be ~1.4x increased in the head-region of obese subjects (Fig. 3A). This was associated with a significantly increased average β-cell cluster area (Fig. 3C). Also, the α-cell area was ~1.7x increased in the head-region of obese subjects compared to lean controls (Fig. 3D). β-Cell and α-cell proliferation were rarely observed (data not shown). The α- to β-cell ratio throughout the pancreas was similar between lean and obese individuals (Fig. 3E).

Discussion

The main results of our study indicate that obesity is associated with adaptation of both β- and α-cell mass and that this adaptation is topologically heterogeneous between different regions in the pancreas.

In line with previous studies (3–7), we found that obesity in humans is associated with an increase of the β-cell mass, most likely to compensate for the increased demand for insulin (14, 15). Also, a strong positive correlation between β- and α-cell mass was observed, which is in accordance by the study of Henquin and Rahier (11). We now show for the first time that the α-cell mass is also increased in obese subjects. Recently it was found that in overweight insulin-resistant non-human primates the fractional α-cell area was significantly increased, and that the changes in α-cell area preceded changes in β-cell area (16). Whether an increased α-cell mass during obesity is a physiological adaptive response to maintain an adequate hormonal balance between insulin and glucagon or that this increase predisposes obese individuals for the development of type 2 diabetes remains an open question (17).

Heterogeneity of the β-cell area throughout the pancreas is well known (6, 18, 19) and in line with these studies we also observe the highest β-cell area in the tail-region of the pancreas. Reers et al. (19) noted an increased islet density in the tail-region when analysing 20 donors with BMIs ranging from 17.2 to 33 kg/m². Here we show that this increased islet density is present in obese donors only. Previous studies have observed fewer α-cells in the part of the pancreas that originates from the ventral bud during embryonic development (20, 21). We now show that, within the part of the human pancreas that is derived from the dorsal bud, the α-cell area is higher in the tail-region.

We recently demonstrated that high-fat diet induced insulin resistance leads to topologically heterogeneous β-cell adaptation in the pancreas of mice (10). Now we show that also in human pancreas β- and α-cell adaptation are topologically heterogeneous.

Interestingly, both β- and α-cell area were increased in the head-region of the pancreas in obese compared to lean subjects. Recently, Wang et al. (22) observed a preferential loss of β-cells in the
pancreatic head-region of patients with type 2 diabetes compared to healthy controls. Together these data suggest that preservation of the endocrine cell mass in the head-region of the pancreas may be of importance for maintenance of normoglycemia in humans. In most histological studies of α and/or β-cell adaptation the head-region of the human pancreas was not included (4–7, 11), which may have led to an underestimation of actual changes in these studies.

The α- to β-cell ratio was found to be negatively correlated with age. This confirms the observation by Rahier et al. (11) that the negative correlation between α-cell mass and age was stronger than for β-cell mass and age in humans. Furthermore, Saisho et al. (5) showed that ageing in humans was not related to loss of β-cell mass. Together these results indicate that with advanced age the β-cell mass is more constant than the α-cell mass. Whether this is a physiological adaptation to counteract the age-associated deterioration in glucose tolerance, which is associated with increased glucagon concentrations (23), remains to be determined.

Following adaptation of the β- and α-cell mass in obesity, the α- to β-cell ratio was preserved. This is in line with the study by Rahier et al. (11) who found a similar α-to-β-cell ratio when comparing subjects with a BMI lower or higher than 25 kg/m². We now show that this ratio is also similar throughout different pancreatic regions in both obese and lean subjects. It has been described that human islets have a unique architecture in which heterologous contacts between β- and α-cells are preferred (24). Insulin secretion from individual human β-cells is enhanced when they are coupled to an α-cell (25), possibly due to paracrine cholinergic stimuli secreted by α-cells (26).

Altogether, our data show that both β- and α-cell mass are increased in obese subjects and that this adaptation is topologically heterogeneous. The α- to β-cell ratio is similar throughout the pancreas and preserved following adaptation in non-diabetic obese subjects.
Supplemental data

<table>
<thead>
<tr>
<th>Lean (BMI&lt;24)</th>
<th>Obese (BMI&gt;27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>Age (years)</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>14</td>
<td>68</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>AVG</td>
<td>53.2</td>
</tr>
<tr>
<td>SEM</td>
<td>3.3</td>
</tr>
</tbody>
</table>


Supplemental figure 1.
Relationship between body mass index and pancreas weight of 154 pancreas donors.
Topologically heterogeneous β- and α-cell adaptation in obese humans

References
