



# Human Islet Morphology Revisited: Human and Rodent Islets Are Not So Different After All

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## Abstract

There has been great interest in understanding how human islets differ from rodent islets. Three major issues about human islet morphology have remained controversial over recent decades: 1) the proportion of the islet made up of  $\beta$ -cells; 2) whether islet cell types have a non-random mantle-core pattern, as seen in rodents, or are randomly scattered throughout the islet; 3) the relation of the different cell types to the blood vessels within the islet, which has implications for inraislet function. We re-examined these issues on immunostained sections of non-diabetic adult human pancreas. The composition of the islets can vary by the analysis method (number vs volume) and by the sampling of islets by size. The majority of adult human islets have clear, non-random clustering of  $\beta$ -cells and blood vessels that penetrate into the  $\beta$ -cell cores. We conclude that although there is far more variability in islet composition both within each human pancreas and among different human pancreas than in rodent pancreas, the islet architecture is not so different between the species. The intrapancreatic variability raises important questions about how islets evolve and function throughout life and how this might relate to the pathogenesis of diabetes. (*J Histochem Cytochem* 63:604–612, 2015)

## Keywords

human islets, islet composition, islet architecture, mantle-core

## Introduction

As the incidence of both type 1 and type 2 diabetes has increased, there has been great interest in the structure and function of human islets and how they differ from rodent islets, which have provided so much of our knowledge about islet biology. The importance of this understanding for human disease is obvious. One would think that there would be general agreement about human islet morphology; however, this is not the case. Three major issues have remained controversial over recent decades. The first is the proportion of the islet that is  $\beta$ -cells; the second is whether there is a variation of the non-random mantle-core pattern seen in rodents or whether different cell types are simply scattered throughout the islet; the third is the relation of the different cell types to the blood vessels

within the islet, which has implications on inraislet function. In this paper, we have revisited these issues by providing both a review of the relevant studies and presenting our own data.

The adult human islet is far more variable than that of rodents: occasional large islets (greater than 200  $\mu$ m diameter) are seen with a majority of glucagon-positive cells (Bonner-Weir et al. 2014; Rahier et al. 1983; Yoon et al. 2003), and islets from the pancreatic polypeptide (PP)-rich

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uncinate process are mainly PP cells, with  $\beta$ -cells making up only  $32.7\% \pm 7.8\%$  as compared with  $65.5\% \pm 4.9\%$  in the rest of pancreas (Sakuraba et al. 2002). Islets from adult non-diabetic humans have been reported to be between 52%–75%  $\beta$ -cell, with some of the discrepancy due to whether the percentage was based on cell number or cell volume (Pisania et al. 2010). With brightfield analysis assessing cell volume, islets have been determined to comprise  $52.0\% \pm 4.1\%$  (Butler et al. 2003),  $59\% \pm 10.3\%$  (Yoon et al. 2003) or 74.8% (Maclean and Ogilvie 1955)  $\beta$ -cells, but, more recently, using laser-scanning confocal microscopy, the  $\beta$ -cell proportion of islets was estimated to be  $53.9\% \pm 2.5\%$  (cell volume, 32 islets isolated from 6 pancreas; Brissova et al. 2005) or 55% (cell number with visible nucleus, 2–5 islets/section, 3 sections/region, 4 regions/5 pancreas; Cabrera et al. 2006). In our studies using ultrastructural analyses to determine cell type and cell boundaries, we found  $73.6\% \pm 1.7\%$   $\beta$ -cells by cell number in islets isolated from 33 pancreases (Pisania et al. 2010).

In most mammalian species, the different islet endocrine cells have a non-random pattern, with a core of  $\beta$ -cells surrounded by a discontinuous mantle of non- $\beta$ -cells that are one to three cells thick (Erlandsen et al. 1976; Orci and Unger 1975). However, humans and some primates have a more complex arrangement, which has led to some reports of a random distribution. Four decades ago, it was suggested that human islets could be considered as composites of several mantle-core subunits or as lobulated with mantle-core lobules (Erlandsen et al. 1976; Orci and Unger 1975). Most non- $\beta$ -cells are found along blood vessels that penetrate the islet and at the islet periphery (Erlandsen et al. 1976; Grube et al. 1983), thus maintaining a mantle-core arrangement. More recently, studies using confocal microscopy have both agreed and disagreed with the concept of human islets having a mantle-core architecture (Bosco et al. 2010; Brissova et al. 2005; Cabrera et al. 2006; Kilimnik et al. 2012; Wang et al. 2013). Brissova et al. (2005) stated, "...[human islets] lack the typical core/mantle...[ $\alpha/\beta$ -] cells were dispersed throughout the islet. This pattern of cell distribution was present in all human islet preparations and islets of various sizes and was also seen in histological sections of human pancreas." Agreeing with them, Cabrera et al. (2006) stated, "Contrary to descriptions of prototypical islets in textbooks and in the literature, human islets did not show anatomical subdivisions. Insulin-immunoreactive cells, glucagon-immunoreactive cells, and somatostatin-containing cells were found scattered throughout the human islet. Human  $\beta$ -cells were not clustered..." However, Bosco et al. (2010) found that, "In small human islets (40–60  $\mu\text{m}$  in diameter),  $\beta$ -cells had a core position,  $\alpha$ -cells had a mantle position, and vessels laid at their periphery. In bigger islets,  $\alpha$ -cells had a similar mantle position but were found also along vessels that penetrate and branch inside the islets." Hara's group reported, "In human islets, while small islets show similar cellular composition

with mice, in larger islets ( $>60 \mu\text{m}$  in diameter), the fraction of  $\alpha$ - and  $\delta$ -cells increases..." and while, "more abundant  $\alpha$ -cells are intermingled with  $\beta$ -cells in the islet core as well as periphery, they are clearly not a random mixture of  $\alpha$ - and  $\beta$ -cells" (Kilimnik et al. 2012; Wang et al. 2013).

In rodents we have previously reported that the islet microvasculature imposed a directional flow of blood through the islet (Bonner-Weir and Orci 1982; Samols et al. 1986) and that the polarity of the  $\beta$ -cells in relation to the capillaries provided an anatomical basis for a functional compartmentalization of the islet (Bonner-Weir 1988). With passive neutralization experiments Samols et al. (1988) showed that directional blood flow was important for regulation of the intraislet interactions, not only in the rat but also in dog, monkey and human (Stagner and Samols 1992; Stagner et al. 1988; Stagner et al. 1992). Yet, more recent studies have suggested that the relation of the blood vessels and islet endocrine cells was different in the human islet than in rodent (Bosco et al. 2010; Cabrera et al. 2006). Cabrera et al. (2006) stated, "Most  $\beta$ -,  $\alpha$ - and  $\delta$ -cells were aligned along blood vessels with no particular order or arrangement, indicating that islet microcirculation likely does not determine the order of paracrine interactions... Thus, an anatomical basis for an order in paracrine signaling as determined by the direction of blood flow likely does not exist for human islets." Moreover, Bosco et al. (2010) reported that, "after a triple immunofluorescence for insulin, glucagon, and CD34, we confirmed that no vascular channel penetrated the core of 40 to 60  $\mu\text{m}$ -diameter islets or the clusters of  $\beta$ -cells in bigger islets."

These three major issues about the architecture of human islets have remained controversial over several decades. We have reexamined the *in situ* islet in the normal human pancreas with these issues in mind. The first is the proportion of the islet occupied by  $\beta$ -cells; the second is whether there is a variation of the mantle-core pattern seen in rodents; the third is the relation of the different cell types to the blood vessels within the islet.

## Materials & Methods

### Tissue

Pancreases from brain-dead donors for clinical islet transplantation were obtained by the Harvard/Joslin Islet Resource Center from the New England Organ Bank following informed consent from the donors' families. Research consent had been obtained for all donors used in this study; the tissues were de-identified and were considered exempt by the Joslin IRB. Only pancreases with cold ischemia at times of 12 hr or less were processed for clinical islet transplantation. For each pancreas, a small piece from the body of the pancreas near the cannulated duct was fixed in 4% (para)-formaldehyde overnight and embedded in

paraffin for quality control assessment; sections from these blocks have been used for the current study.

### Immunostaining

Sections were immunostained for insulin (guinea pig anti-porcine insulin, 1:200, Linco or 1:1000, Dako; Carpinteria, CA), glucagon (rabbit anti-bovine glucagon; 1:3000, gift from Dr. M. Appel, UMass Medical Center, Worcester, MA) and somatostatin (rabbit anti-somatostatin, 1:200, EMD Millipore; Billerica, MA). The sections were incubated overnight at 4°C, washed with Tris buffer, pH 7.4, sequentially incubated with goat anti-rabbit IgG and rabbit peroxidase anti-peroxidase (PAP) (Cappel Laboratories; Cochranville, PA), reacted with chromagen 3,3'-diaminobenzidine (DAB, Sigma-Aldrich; St Louis, MO) and counterstained with hematoxylin. Alternatively, sections were incubated with a mixture of the anti-glucagon and anti-somatostatin antibodies overnight and then anti-insulin antibodies for 1 hr at room temperature, followed by biotinylated secondary antibodies (biotinylated anti-rabbit IgG, AlexaFluor488-streptavidin; AlexaFluor594 anti-guinea pig IgG, all at 1:200 and all from Jackson ImmunoResearch Laboratories; West Grove, PA) for 1 hr at room temperature. Since the tissues were from the body and pancreatic polypeptide (PP) poor areas, we did not include anti-PP antibody staining. To visualize vasculature relationships with the endocrine cells, endocrine cells were stained with insulin and glucagon antibodies as above and blood vessels with a mixture of mouse antibodies against smooth muscle actin (1:4000; Sigma-Aldrich) and CD34 (1:500, Biogenex; Fremont, CA) (Cabrera et al. 2006) with an amplification by tyramide kit (Perkin-Elmer; Fremont, CA). Slides were mounted with Vectashield with DAPI (Vector Laboratories; Burlingame, CA) for nuclear staining. Immunostained sections were examined using an Olympus BH-2 microscope (Olympus, Tokyo, Japan) or in confocal mode on a Zeiss LSM 710 microscope (Zeiss Microimaging; Thornwood, NY). Figures were compiled using Adobe Photoshop (Adobe Systems, Inc.; San Jose, CA).

### Quantification

For islet composition, glucagon immunoperoxidase-stained sections from seven consecutive donor pancreases with research consent were systematically scanned under 40× objective. For composition, at least 25 islets with 30 or more cells in cross section were counted at a final magnification of 420× for each donor; this comprised at least 2400 cells (with at least 800 in islets between 30–99 cells). After quantification, the islets were binned as medium (30–99 cells, 50–175 μm in diameter) and large (greater than 100 cells or 175 μm in diameter).

Using the same immunostained slides, all islets on the section were assessed for the clustering of β-cells that resembled the mantle-core subunit pattern found in rodents. After being categorized as having or not having clumps of β-cells, the islets were categorized by the number of cells/cross section: small (20–30 cells), medium (30–99 cells) and large (greater than 100 cells).

Cell composition by histological brightfield determination of the quality-controlled pancreatic sections (as above) was compared to that of ultrastructural analysis of the isolated islet preparation (Pisania et al. 2010) for an additional seven donors that were consecutively used for clinical transplants. With electron microscopy (EM), acinar cells, islet cells (β-, and the non-β cells α, δ and PP), and duct cells can be definitively identified, and cell composition determined by counting cells to give the proportion of the different cell types (Pisania et al. 2010).

### Statistical Analysis

Two tailed student's *t*-test was used for statistical analysis with *p*<0.05 considered significant.

## Results

### Islet Composition

Human islet composition by cell type has been reported numerous times but with major differences in the reported values. These differences could be due to methodological and/or sampling differences. We have previously reported a mean of 73.6% ± 1.7% β-cells/islet from 33 pancreases for clinical islet transplantations in the Harvard program (Pisania et al. 2010) using numeric quantification of ultrastructurally identified islet cell types. Here, we compared the composition of seven consecutive isolations that were used for clinical transplants with two different methods to quantify the number of cells; first, at the ultrastructural level and second, using immunoperoxidase staining on quality-controlled section (Table 1). By ultrastructure, β-cells accounted for 72.2% ± 3.5% of the islet cells and, by immunostaining, 70.3% ± 3.0%. By the ultrastructural analysis, all islet cells were counted without regard to whether they were in large islets or as single cells, whereas in the immunostained sections, we arbitrarily considered 40 μm as the minimum diameter for a cluster of islet cells to be considered an islet. Such an islet was typically 20–30 endocrine cells in cross section. Although the above mean values do not differ, the values for the same donor were variable with the two methods, suggesting that there was a difference due to sampling, since only 500–700 islet cells were counted for the ultrastructural analysis.

We then assessed the cell composition by islet size in another subset of donor pancreases using immunoperoxidase

**Table 1.** Comparison of Proportion of  $\beta$ -cells in Human Islets of Same Donors using Two Different Cell Counting Methods.

Donor	Age (Years)	Sex	BMI	Total Cells (Using EM)	$\beta$ -Cell/Islet (%)	
					Using EM	Using LM
H0424	54	M	25.4	621	83.9	66.5
H0415	56	F	36.9	629	57.1	76.5
H0411	57	F	21.4	558	73.5	73.9
H0410	58	M	25.2	548	65.5	56.3
H0407	50	F	47.6	669	71.9	66.9
H0406	54	M	28.3	662	82.7	79.9
H0401	38	F	20.8	791	70.8	72.3
				<b>Mean (SEM)</b>	<b>72.2 (3.5)</b>	<b>70.3 (3.0)</b>

BMI, body mass index; M, male; F, female; EM, electron microscopy; LM, light microscopy

**Table 2.** Cell Composition of Human Islets in Islets of Different Sizes.

Donor	Age (Years)	Sex	BMI	Counted Cells	Islets	More Than 100		30–99	
						Cells/Islet	$\beta$ -Cells (%)	Cells/islet	$\beta$ -Cells (%)
H0015	15	M	25.1	2402	23	225	45.5	51.5	74.1
H0017	23	M	26.0	3354	35	159	65.5	62.8	76.6
H0018	48	F	17.9	3137	30	161	56.1	66.7	72.3
H0019	64	F	20.8	4146	36	189	64.2	56.0	68.2
H0020	16	M	32.5	2624	27	164	59.7	51.4	70.2
H0021	39	F	22.5	3108	34	154	60.6	57.1	77.4
H0022	53	F	23.5	3537	35	186	67.7	56.9	80.6
<b>Mean <math>\pm</math> SEM</b>					<b>31.4 <math>\pm</math> 1.9</b>	<b>176.9 <math>\pm</math> 9.5</b>	<b>59.9 <math>\pm</math> 2.8*</b>	<b>57.5 <math>\pm</math> 2.1</b>	<b>74.2 <math>\pm</math> 1.6*</b>

\*Two-tailed unpaired *t*-test; significance;  $p < 0.0019$ . BMI, body mass index; M, male; F, female; 30–99 cells represents islets that are 60–175  $\mu$ m in diameter; 100+ cells represents islets that are  $< 175$   $\mu$ m in diameter.

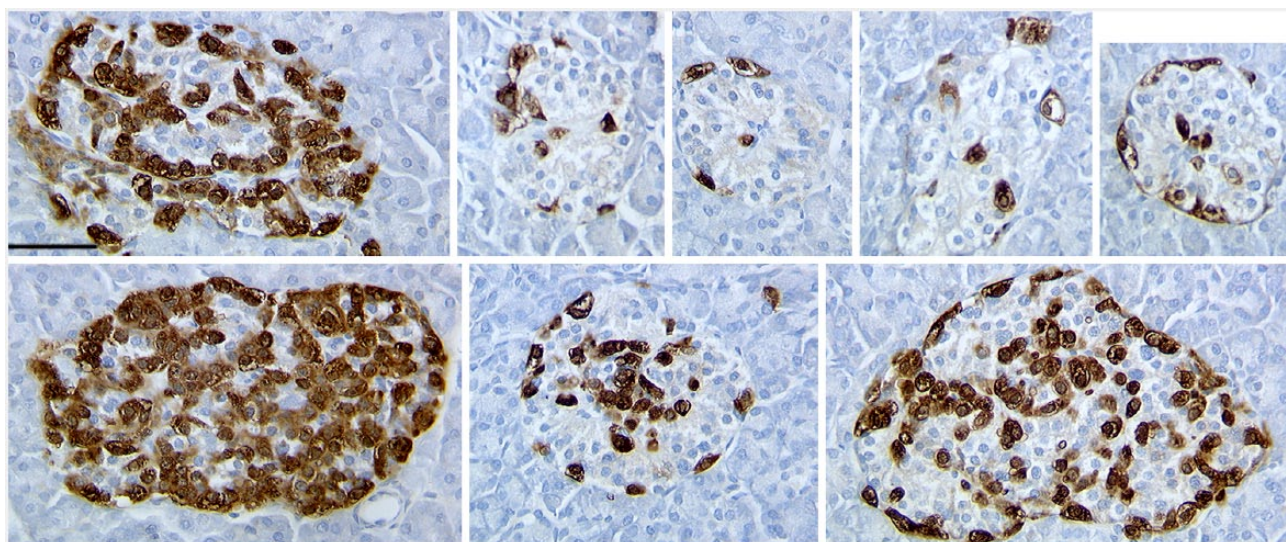
staining (Table 2); this cohort consisted of seven consecutive donors with research consent. With systematic scanning of the sections, all islets (at least 50  $\mu$ m in diameter) were counted until at least 25 islets or 2500 cells were assessed (with the exception of H0015, in which counting all the islets greater than 30 cells on the section did not quite reach these cutoffs). In all but one case (H0019), the islets greater than 100 cells in cross-section (greater than 175  $\mu$ m in diameter) had a significantly lower proportion of  $\beta$ -cells/islet than the medium-sized islets. The large islets (mean, 177  $\pm$  10 cells) had a mean of 59.9%  $\pm$  2.8%  $\beta$ -cells/islet, and the medium-sized islets (mean, 57.5  $\pm$  2.1 cells) had 74.2%  $\pm$  1.5%  $\beta$ -cells/islet (two-tailed paired *t*-test,  $p < 0.003$ ). Strikingly, as has been previously reported (Bonner-Weir et al. 2014; Butler et al. 2013; Yoon et al. 2003), there were some large islets that had only a small proportion of  $\beta$ -cells, and were instead mainly composed of glucagon-staining cells [see Fig. 1 (H0021) and Fig. 2 (H015)].

### Organization of Islets

Whereas others have done complex analyses of cell-to-cell contacts to conclude that human islets were “clearly not a

random mixture of  $\alpha$ - and  $\beta$ -cells” (Kilimnik et al. 2012; Wang et al. 2013), we have been struck by the clear patterns on glucagon-stained sections as illustrated in Figure 3. Larger islets usually consist of multiple subunits, whereas smaller islets resemble the classic rodent pattern. In the set of seven consecutive donors shown in Table 2, we analyzed all islets (40  $\mu$ m or greater diameter) as to whether they had a clear pattern of mantle-core with sizable clusters of  $\beta$ -cells partially surrounded by non- $\beta$ -cells. Table 3 shows the proportion of islets that lacked an obvious clustering of  $\beta$ -cells surrounded by non- $\beta$ -cells. In five pancreases, less than 10% of the islets lacked an obvious mantle-core pattern, and only in 2/7 (28.5%) of the pancreases were there a large number of islets without the obvious mantle-core architecture. Even those islets classified as lacking mantle-core architecture did indeed have small clusters of  $\beta$ -cells and tended to have a higher proportion of non- $\beta$ -cells than the others.

The pattern seemed inherent to the individual pancreas, as islets of different size categories had similar values within a donor pancreas yet there were marked differences among the donors. Another striking variable was the distribution of islet sizes among the different donors, as noted in Table 3, since all islets per section were assessed.



**Figure 1.** Representative islets in pancreas from donor H0015 immunostained for glucagon (brown) showed great variability in islet composition, both in the proportion of cell types and the clarity of  $\beta$ -cell core arrangement. The smaller islets resemble those of rodents with a clear mantle-core pattern. However, as shown here, even the larger islets with fewer  $\beta$ -cells have a discernible clustering of  $\beta$ -cells. Scale, 50  $\mu$ m.

### *Relationship to Intra-islet Blood Vessels and Its Implications*

The islet microvasculature in rodents is thought to provide a functional compartmentalization for the interactions of islet cells (Weir and Bonner-Weir 1990). However, the random pattern of islet cell types in human islets purported to be present by some groups would preclude such regulated local paracrine effects. It is of interest that Bosco et al. (2010) defined a 3-dimensional visualization of the human islets based on “trilaminar epithelial plates [42  $\mu$ m thick], folded with different degrees of complexity and bordered by vessels on both sides.” Yet they reported that “after a triple immunofluorescence for insulin, glucagon, and CD34, ... no vascular channel penetrated the core of 40 to 60  $\mu$ m-diameter islets or the clusters of  $\beta$ -cells in bigger islets.” This finding seemed odd to us based on rodent islets, so we examined whether human islets about 50  $\mu$ m in diameter had internal blood vessels and whether there were blood vessels penetrating into the  $\beta$ -cell cores of larger islets.

In many of the brightfield images of immunostained sections (Figs. 2 and 3), vascular channels or vessels can be seen coursing in larger islets. In immunofluorescence images stained for islet hormones (Fig. 4), these vascular channels appear as black spaces between cords of cells. However, to clearly visualize the blood vessels (CD34+) and their pericyte/smooth muscle cells (smooth muscle actin+, SMA) that fill these vascular channels, we immunostained pancreas sections using antibodies against insulin, glucagon and a mixture of antibodies against CD34 and

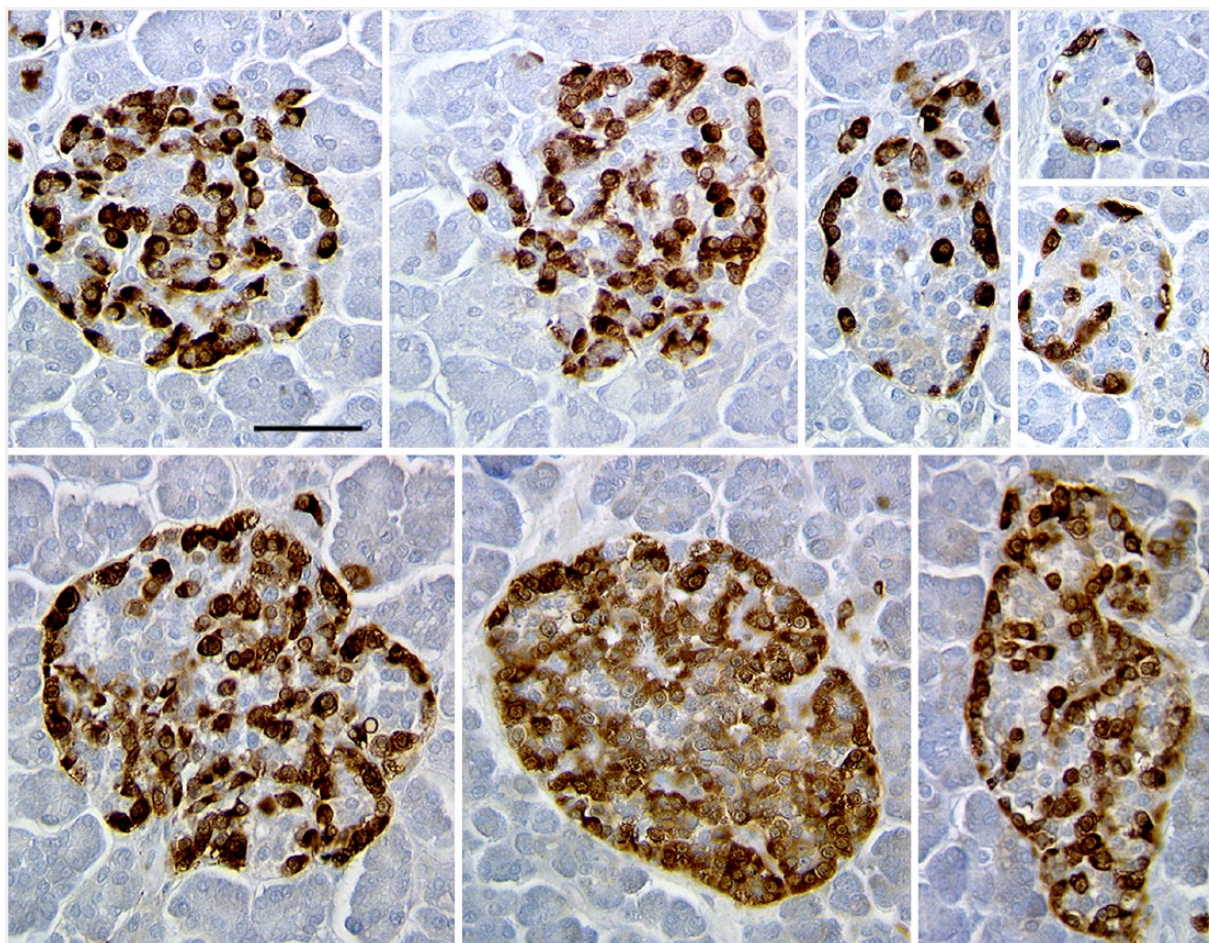
SMA. As shown in Figure 5, blood vessels clearly penetrate into the clusters of  $\beta$ -cells and into the centers of small 50- $\mu$ m diameter islets. Indeed, the pattern of  $\beta$ -cells adjacent to the blood vessels is often in the rosette pattern we described in rat islets (Bonner-Weir 1988).

### **Discussion**

After a re-examination of the architecture of the human islet, we conclude that the human islet is similar to the rodent islet in terms of the three controversial issues investigated. Even so, there is far more variability in composition within each human pancreas as well as among different human pancreas than seen in rodent pancreas. This variability necessitates care in sampling islets both as to the number and size for both morphological and functional studies. Importantly, it raises questions about how human islets evolve and function throughout life and how this might relate to the pathogenesis of diabetes.

The issue of islet cell composition is particularly sensitive to the method of determination, (whether by volume or number) and to the size of the islets. We previously discussed the issue of volume versus number (Pisania et al. 2010). The large difference in size/volume of the different cell types results in the discrepancies obtained by assessments of volume and cell number. Most notably, the  $\beta$ -cells are larger and so are more likely to be cut in a sectioned islet than would be the case for the smaller  $\alpha$ - or  $\delta$ -cells. There are additional issues including whether the volume only comprises hormone-positive cells or the whole islet, including vascular channels, and whether only cells with clear





**Figure 2.** Representative islets in pancreas from donor H0021 immunostained for glucagon (brown) showed greater variability in islet composition, both in the proportion of cell types and the clarity of  $\beta$ -cell core arrangement. Some of the large islets have mainly glucagon staining cells, with few  $\beta$ -cells (here unstained). Scale, 50  $\mu$ m.

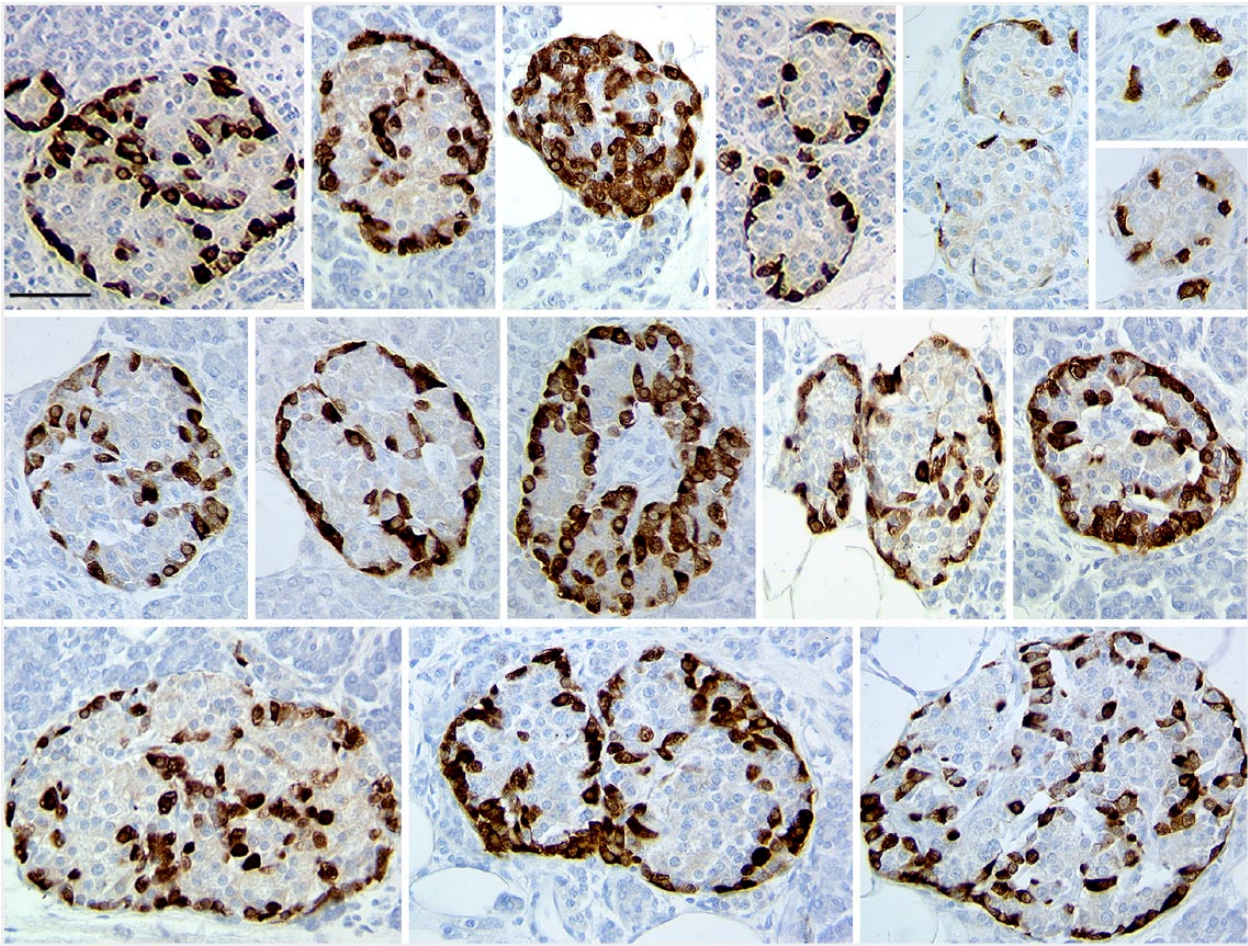
nuclei are counted. Thus, some caution is needed when comparing the proportions of cell types between different studies. In the current study, we found that cell composition varied with islet size. As seen in Table 2, larger islets had a lower percentage of  $\beta$ -cells being only  $59.9\% \pm 2.8\%$ , whereas medium-sized islets were  $74.2\% \pm 1.6\%$  even in the same sections. These latter islets were not the tiny clusters often seen that would not contribute much to the total  $\beta$ -cell mass but rather the bulk of the islets within the pancreas, with a mean of 57.5 cells in cross section or about 120  $\mu$ m in diameter. Moreover, the size distribution of islets varied among different adult human pancreases.

As far as the presence of non-random organization of the human islets, we conclude that the majority of islets do have the pattern of composites of modular subunits that resemble the rodent mantle-core arrangement. It is particularly striking that, in all the pancreases studied, the small islets closely resembled the rodent mantle-core pattern as previously noted (Bosco et al. 2010; Kilimnik et al. 2012; Wang et al.

2013). Most of the larger islets consisted of subunits of cell aggregates with a mantle-core arrangement similar to rodent islets. However, some of the larger islets with a lower proportion of  $\beta$ -cells gave the suggestion of random organization. Yet even in these larger islets, there was clear clustering of  $\beta$ -cells, just smaller clusters.

The third issue was the relation of the islet cells and the blood vessels. It is clear to us that blood vessels penetrate both into the  $\beta$ -cell clusters in larger islets and into centers of small islets, unlike the conclusions drawn in the study of Bosco et al. (2010). Their lack of finding such vessels may be due to a technical problem. When we used only immunostaining for CD34 to visualize the vessels—as they did—it was difficult to see any vessels within the islets, even in putative vascular channels, whereas the vessels in the exocrine pancreas were stained. This difficulty may be due to lower CD34 expression in the fenestrated islet capillaries than in non-fenestrated capillaries in the surrounding exocrine tissue. However, using a mixture of





**Figure 3.** Representative islets in pancreas from donor H0019 immunostained for glucagon (brown) showed a clear pattern of small subunits resembling that of the rodent mantle-core islet organization in most of the islets of all sizes. Scale, 50  $\mu$ m.

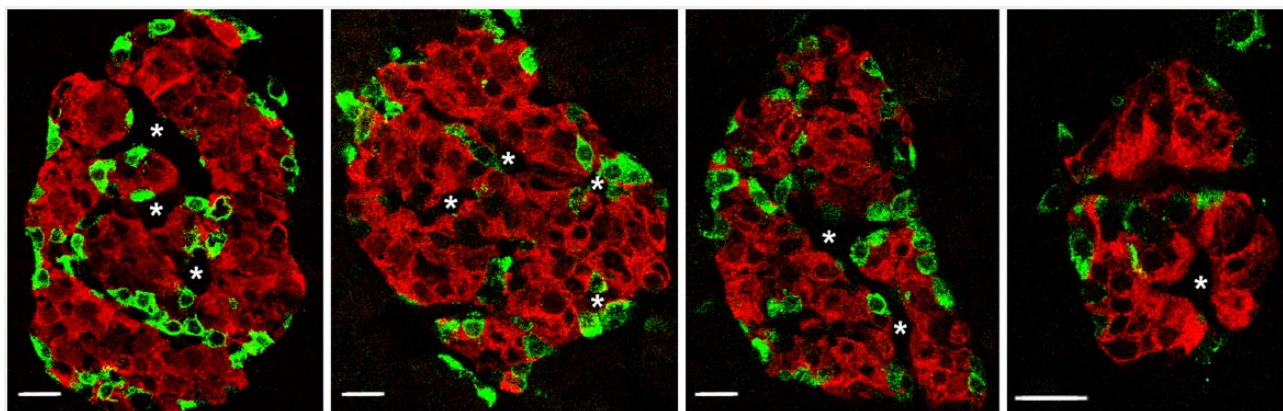
**Table 3.** Percentage of Human Islets within a Pancreatic Section without Obvious  $\beta$ -cell Cored Subunits.

Donor	Large	Medium	Small	Total Islets Counted	Large (%)	Medium (%)	Small (%)
H0015	9/15	7/25	5/10	50	60.0	28.0	50.0
H0017	5/30	16/47	9/37	104	16.7	34.0	24.3
H0018	3/42	2/34	1/18	94	7.1	6.0	5.6
H0019	0/35	7/154	6/85	274	0.0	5.9	7.1
H0020	0/12	2/22	1/43	77	0.0	4.5	2.3
H0021	1/18	6/66	1/44	128	5.5	9.1	2.3
H0022	0/26	1/34	1/20	80	5.0	2.9	5.0
<b>Mean <math>\pm</math> SEM</b>				<b>115 <math>\pm</math> 28</b>	<b>13.5 <math>\pm</math> 7.4</b>	<b>12.9 <math>\pm</math> 4.4</b>	<b>13.8 <math>\pm</math> 6.2</b>

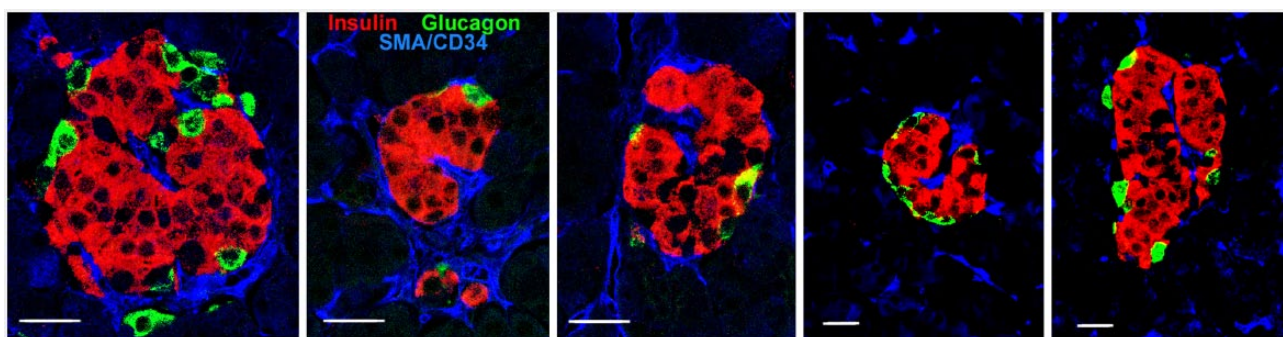
antibodies against smooth muscle actin and CD34, as used by Cabrera et al (2006), we could see staining along the putative vascular channels since the pericytes or smooth muscle cells surrounding the vessels were also stained. A clear intraislet vascularization, even in the small islets, makes sense since our understanding is that  $\beta$ -cells have

high oxygen needs for proper function. In fact, with hypoxia  $\beta$ -cells secrete VEGF to enhance their vascularization (Vasir et al. 1998; Vasir et al. 2000). Together, the mantle-core subunit arrangement and the intraislet vessels penetrating the  $\beta$ -cell cores support the physiological studies of Stagner and Samols (1992) on intraislet interactions in the





**Figure 4.** Islets in pancreas from donor H0021 immunofluorescently stained for insulin (red) and non- $\beta$ -cells (glucagon and somatostatin; green) show clear clustering of  $\beta$ -cells with surrounding non- $\beta$ -cells. Additionally, apparent vascular channels (white asterisks) are seen penetrating in the  $\beta$ -cell clusters. Scale, 20  $\mu$ m.



**Figure 5.** Even small islets of pancreas from donor H0021 show blood vessels, here visualized by immunostaining for smooth muscle actin and CD34 (blue), coursing into clumps of  $\beta$ -cells (red) with no glucagon cells (green) nearby. Scale, 20  $\mu$ m.

human islet being similar to that in rodents. In conclusion, whereas human and rodent islets may have some functional differences—many to still be determined—their fundamental architectural arrangement is similar.

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