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Islet Microvasculature Alterations With Loss of Beta-cells in Patients With Type 1 Diabetes

Joseph S. Canzano, Lith H. Nasif, Elizabeth A. Butterworth, Dongtao A. Fu, Mark A. Atkinson, and Martha Campbell-Thompson
Department of Pathology, Immunology and Laboratory Medicine (JSC, LHN, EAB, DAF, MAA, MC-T); and Department of Pediatrics, College of Medicine (MAA), University of Florida, Gainesville, Florida

Summary
Islet microvasculature provides key architectural and functional roles, yet the morphological features of islets from patients with type 1 diabetes are poorly defined. We examined islet and exocrine microvasculature networks by multiplex immunofluorescence imaging of pancreases from organ donors with and without type 1 diabetes (n=17 and n=16, respectively) and determined vessel diameter, density, and area. We also analyzed these variables in insulin-positive and insulin-negative islets of 7 type 1 diabetes donors. Control islet vessel diameter was significantly larger (7.6 ± 1.1 μm) compared with vessels in diabetic islets (6.2 ± 0.8 μm; p<0.001). Control islet vessel density (number/islet) was significantly lower (5.3 ± 0.6) versus diabetic islets (9.3 ± 0.2; p<0.001). Exocrine vessel variables were not significantly different between groups. Islets with residual beta-cells were comparable to control islets for both vessel diameter and density and were significantly different from insulin-negative islets within diabetic donors (p<0.05). Islet smooth muscle actin area had a significant positive correlation with age in both groups (p<0.05), which could negatively impact islet transplantation efficiency from older donors. These data underscore the critical relationship of islet beta-cells and islet vessel morphology in type 1 diabetes. These studies provide new knowledge of the islet microvasculature in diabetes and aging. (J Histochem Cytochem 67:41–52, 2019)

Keywords
alpha-cell, CD3, CD31, CD34, endothelium, exocrine, glucagon, insulin, microenvironment, secretogranin 3, smooth muscle actin

Introduction

Type 1 diabetes is a chronic disorder resulting from complex genetic, environmental, and immunological factors that drive autoimmune responses to islet beta-cell antigens resulting in the irreversible loss of beta-cell mass.[1,2] These destructive processes begin months to years before clinical (i.e., symptomatic) onset of the disease. Ongoing autoimmunity and beta-cell dysfunction and destruction are asymptomatic during this prodromal period but can be identified serologically by the presence of autoantibodies against one or more beta-cell autoantigens and metabolically by the loss of first-phase insulin secretion in response to glucose.[3,4] After clinical onset, a partial remission phase (so-called "honeymoon") can occur where the patient may require less insulin to maintain glycemic control.[5,6] This phase has been hypothesized to represent a critical window for inducing adult beta-cell

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Corresponding Author:
Martha Campbell-Thompson, Department of Pathology, Immunology and Laboratory Medicine, University of Florida, 1395 Center Drive, Gainesville, FL 32610-0275, USA.
E-mail: mct@ufl.edu

regeneration and/or replacement in conjunction with anti-autoimmune strategies to prevent further beta-cell losses. An improved understanding of the pathological processes occurring within islets containing residual beta-cells would aid in the design of therapeutic strategies after onset of diabetes.

The islet microvasculature is organized into a glomerular-like network of highly fenestrated capillaries surrounded by a unique double basement membrane.[7–13] The islet microvasculature is required for normal endocrine cell development and serves a critical role in hormone secretion.[11,14,15] Pancreatic islets are well perfused, with an estimated blood perfusion rate of 5–15% of the entire organ despite representing only 1–2% of pancreatic volume.[9,14,16] Each islet endocrine cell is in close proximity to the microvasculature, ensuring efficient transfer of nutrients and signaling factors between endocrine and endothelial cells.[17–19] Islet blood flow rate is regulated through complex interactions between the autonomic and sensory nervous systems and paracrine factors from endocrine cells.[7,9] The microvasculature also serves an important barrier function to leukocyte transmigration, and loss of endothelial integrity contributes to inflammation.[14]

Recent studies show that the human islet microvasculature is ~5-fold less dense (number of vessels/islet area) than in mouse islets.[10,20–23] Human islet vessels were reported to contain three-times higher α-smooth muscle actin (SMA) cells compared with islets from mice where SMA cells were found associated only with feeding arterioles found at the islet periphery.[21] Furthermore, patients with type 2 diabetes had increased islet vessel density or area compared with non-diabetic donors.[20,23] The purpose of the current study was to conduct morphometric studies of the islet microvasculature in patients with type 1 diabetes in comparison to matched control donors. Both islet and exocrine microvascular networks were analyzed for vessel diameters, densities, and areas and SMA areas. Islet vessel morphology was also studied in a subset of type 1 diabetes patients with residual beta-cells and another subset of those donors with insulitis in insulin-positive islets.

Materials and Methods

Donors and Pancreas Samples

Formalin-fixed, paraffin-embedded serial sections were provided by the JDRF Network for Pancreatic Organ donors with Diabetes (nPOD) program using protocols previously described for pancreas recovery and immersion fixation.[24] Donors included (1) non-diabetic controls with no history of diabetes or pancreatic disease and negative for type 1 diabetes-associated autoantibodies (control, n=16), and (2) donors with type 1 diabetes (n=17; Table 1). These studies were conducted following approval by the University of Florida Institutional Review Board (521-2008) under an exempt status for cadaveric studies. Serial paraffin sections (4 μm) were obtained from blocks in the body-tail region previously screened by H&E and IHC.[25]

Multiplex Immunofluorescence

Multiplex immunofluorescence was conducted using 4 primary antibodies to detect all neuroendocrine cells: secretogranin 3 (SCG3), beta-cells (insulin), vascular endothelium (CD34, CD31), and SMA following methods previously described[27] (Table 2). Control stains showed that islet vessels were uniformly labeled by both CD34 and CD31 (Supplemental Fig. 1). Paraffin sections were dewaxed and rehydrated with TBS and with 0.05% TBST. Nonspecific binding was blocked by incubation in 10% goat serum in TBST for 1 hr at room temperature. Sections were incubated in primary antibodies to SCG3 for 1 hr at room temperature followed by tyramine signal amplification (TSA) with Opal 520 (PerkinElmer; Waltham, MA) conjugate followed by anti-CD34 for 1 hr at room temperature and TSA with Opal 670 conjugate. Sections were reblocked before incubation with anti-insulin antibody overnight at 4C, followed by TBST washes and incubation with goat antiguinea pig Alexa 405 (ThermoFisher Scientific; Waltham, MA) for 1 hr at room temperature. Tissues were reblocked and mouse anti-SMA-Cy3 antibody applied overnight at 4C. Sections were mounted with ProLong Gold Antifade (Life Technologies; Grand Island, NY). Additional sections were stained in 4 control donors and 8 donors with type 1 diabetes for SCG3, CD3, glucagon, and insulin and insulitis determined as previously described.[27]

Confocal Microscopy

Photomicroscopy was performed on a Zeiss 710 LSM confocal microscope using the 405, 488, 561, and 633 nm laser lines to excite AlexaFluor 405, Opal 520, Cy3, and Opal 670, respectively. Opal 520 and 670 emissions were collected simultaneously (510–545 nm and 640–750 nm) whereas AlexaFluor 405 (405–470 nm) and Cy3 (560–595 nm) were collected separately to eliminate fluorochrome crosstalk. Sections were photographed and analyzed by one observer (J.C.). The entire pancreas section was scanned with a 10× objective, then islets were randomly selected from multiple lobules after careful evaluation of the entire section using the selection criteria as follows: (1) spherical to

Table 1. Donor Demographics and Beta-cell Analyses.

Case ID	Age (Years)	Diabetes Duration (Years)	Sex	Race	BMI	C-Pep[a]	Cause of Death	AAb[b]	Insulin Area (%)[c]	Insulin Mass (mg)[c]
No diabetes (Control)										
6318	10.0		F	Caucasian	17.6	3.89	Head Trauma	Negative	0.87	262
6358	13.0		M	African American	19.2	8.00	Cerebrovascular/Stroke	Negative	1.16	609
6233	14.0		M	Caucasian	21.9	7.26	Anoxia	Negative	2.62	1596
6282	14.0		M	Caucasian	41.9	6.83	Head Trauma	Negative	3.81	2890
6232	14.0		F	Caucasian	20.8	19.50	Head Trauma	Negative	1.79	886
6099	14.2		M	Caucasian	30.0	5.37	Head Trauma	Negative	1.53	1307
6317	15.0		M	Caucasian	29.8	7.15	Head Trauma	Negative	1.79	796
6271	17.0		M	Caucasian	24.4	11.47	Head Trauma	Negative	1.99	1950
6227	17.0		F	Caucasian	26.4	2.75	Cerebrovascular/Stroke	Negative	1.21	731
6279	19.0		M	Caucasian	34.0	8.01	Head Trauma	Negative	2.79	2237
6238	20.0		M	African American	21.7	1.17	Head Trauma	Negative	1.83	1675
6179	21.8		F	Caucasian	20.7	2.74	Head Trauma	Negative	NA	NA
6057	22.0		M	Caucasian	26.0	16.23	Head Trauma	Negative	1.59	1657
6160	22.1		M	Caucasian	23.9	0.40	Head Trauma	Negative	0.51	403
6333	27.0		F	Caucasian	24.9	9.37	Anoxia	Negative	0.66	453
6091	27.1		M	Caucasian	35.6	7.71	Head Trauma	Negative	0.69	658
Mean ± SD	18.0 ± 5.0		5F/11M		26.2 ± 6.6	7.37 ± 5.1			1.66 ± 0.90	1207 ± 770
Type 1 Diabetes										
6265	11.0	8	M	Caucasian	12.9	0.06	Cerebrovascular/Stroke	$GADA^+$ $mIAA^+$	0.06	10
6264	12.0	9	F	Caucasian	22.0	0.00	DKA, cerebral edema	Negative	0.06	13
6243	13.0	5	M	Caucasian	21.3	0.42	Cerebrovascular/Stroke	$mIAA^+$	0.50	147
6089	14.3	8	M	Caucasian	26.0	0.00	Anoxia	$mIAA^+$	0.00	1
6049	15.0	10	F	African American	20.8	0.00	Anoxia	$GADA^+$ $mIAA^+$	0.05	9
6083	15.2	11	F	Caucasian	18.4	0.00	DKA, cerebral edema	$mIAA^+$	0.00	1
6396	17.1	2	F	Caucasian	22.6	0.06	DKA, cerebral edema	Negative	0.01	4
6237	18.0	12	F	Caucasian	26.0	0.00	Head Trauma	$GADA^+$ $mIAA^+$	0.01	3
6046	18.8	8	F	Caucasian	25.2	0.00	Anoxia	$IA\text{-}2A^+$ $ZnT8A^+$	0.05	17
6195	19.3	5	M	Caucasian	23.7	0.00	Head Trauma	$GADA^+$ $IA\text{-}2A^+$ $ZnT8A^+$ $mIAA^+$	0.02	6
6064	19.6	9	F	Caucasian	22.6	0.00	Anoxia	$GADA^+$ $IA\text{-}2A^+$ $ZnT8A^+$ $mIAA^+$	0.01	5
6325	20.0	6	F	African American	31.2	0.14	Anoxia	$GADA^+$ $IA\text{-}2A^+$ $mIAA^+$	0.31	129
6245	22.0	7	M	Caucasian	23.2	0.00	Head Trauma	$GADA^+$ $IA\text{-}2A^+$	0.55	174
6070	22.6	7	F	Caucasian	21.6	0.00	Anoxia	$IA\text{-}2A^+$ $mIAA^+$	0.09	36
6362	24.9	0	M	Caucasian	28.5	0.38	Head Trauma	$GADA^+$	0.33	176
6321	27.0	16	F	Caucasian	20.3	0.00	Anoxia	$IA\text{-}2A^+$ $ZnT8A^+$ $mIAA^+$	0.00	0
6324	29.0	2	M	Hispanic	26.2	0.00	Anoxia	$GADA^+$ $mIAA^+$	0.00	0
Mean ± SD	18.8 ± 5.2	7.4 ± 3.9	10F/7M		23.1 ± 4.1	0.06 ± 0.13			0.12 ± 0.18	43 ± 66

Abbreviations: BMI, body mass index; DKA, diabetic ketoacidosis; GADA, glutamic acid decarboxylase; mIAA, insulin; IA-2A, insulinoma-associated protein-2; ZnT8A, zinc transporter-8; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.

[a]C-peptide levels (μg/ml).

[b]Autoantibody (AAb) status. All donors had AAb testing by radioimmunoassay (RIA) as previously reported.[26] RIA data values were converted to NIDDK units and defined as positive if one or more of the following applied: GADA if ≥20, IA-2A if >5, $mIAA^+$ if ≥ 0.10, or ZnT8A if >0.020. $mIAA^+$ antibodies may present those resulting from insulin therapy.

[c]Insulin fractional area and mass were previously reported.[27]

Table 2. Antibodies Used for the Analysis of the Human Pancreas Microvasculature.

Antigen	Target	Host	Company	Catalog No.	Dilution
SMA	Smooth muscle cells	Mouse	Sigma; St. Louis, MO	6198	1:200
CD3	T Lymphocytes	Rabbit	Dako; Carpinteria, CA	A0452	1:500
CD31	Endothelial cells	Rabbit	Abcam; Cambridge, MA	AB28364	1:250
CD34	Endothelial cells	Mouse	Life Tech.; Grand Island, NY	MA1-80202	1:300
GCG	Alpha-cells	Mouse	Abcam	AB10988	1:200
INS	Beta-cells	Guinea Pig	Dako	A0564	1:100
SCG3	Neuroendocrine cells	Rabbit	Sigma	HPA006880	1:1000

Secondary antibodies were Opal (PerkinElmer; Waltham, MA) or AlexaFluor (ThermoFisher Scientific; Waltham, MA) conjugates at 1:200–1:1000 dilutions. Abbreviations: SMA, α-Smooth Muscle Actin-Cy3; CD3/31/34, vascular endothelium; GCG, glucagon; INS, insulin; SCG3, secretogranin 3.

oval shape, (2) diameter between ~50–150 μm, and (3) no immediately adjacent duct or large artery. Islets were imaged using a Plan-Apochromat 20× objective (0.8 numerical aperture, 0.15 μm/pixel), maximum resolution, and 1 airy unit pinhole. Numbers of islets and peri-islet regions analyzed per donor are shown in Supplemental Table 1. For the donors with type 1 diabetes, additional islets were imaged with residual beta-cells (INS^+, n=7 donors). INS^+ islets were infrequent and often found within certain lobules and, hence, could not be randomly sampled (Supplemental Table 1). Insulitic islets were further studied in the INS^+ islets (n=5 donors). Insulitic islets were defined as 3 or more islets per section with >5 $CD3^+$ cells/islet, immediately adjacent to or within INS^+ islets as used in a previous study.[27]

Morphometric Analyses

Endocrine and exocrine vessel morphometric analyses were performed using custom Python scripts created for FIJI software (v2.0; https://fiji.sc).[28] Total islet area was identified by thresholding the $SCG3^+$ area and applying morphological filters to acquire a smoothed islet boundary to include all $SCG3^+$, $CD34^+$, and SMA^+ areas (Fig. 1). Islets were categorized for insulin immunopositivity ($INS^{+/-}$) using a background-corrected insulin intensity and visual confirmation for every islet. Islet $CD34^+$ area (%), insulin area (%), and SMA^+ area (%) were determined via thresholding of the immunostained area divided by total islet area. Particle analysis was used to determine $CD34^+$ islet vessel density (vessels/μm^2 islet area) based on previously described methods for individual vessel counts and area (%).[29] The plug-in Geodesic Diameters was used to determine vessel diameters via maximum inscribed circles for each vessel, and average vessel diameter was determined for each islet.[30] Similar calculations were performed in the same image field for the peri-islet exocrine microvasculature excluding islets and major vessels and ducts.

Statistics

Data are expressed as means ± SD with number of donors (N) unless otherwise noted, using 5–35 islets/donor. Unpaired Mann-Whitney U tests were used to compare islet and peri-islet exocrine vessels between control and diabetic donor groups. Wilcoxon matched-pairs signed rank tests were used to compare islet and exocrine vessels within diabetic donors for islet $INS^{+/-}$ and $CD3^{+/-}$ subtypes. All data were compiled in Excel, and statistics were performed using GraphPad Prism v6 (GraphPad Software Inc.; San Diego, CA). Correlations were tested by Spearman's analysis with linear regression analysis. A $p<0.05$ was considered statistically significant.

Results

Islet Vessels Were Smaller and More Numerous in Donors With Type 1 Diabetes

The marker SCG3 labeled the cytoplasmic compartment in all islet neuroendocrine cells and delineated total islet area using the custom script (Fig. 1A–C; SCG3). In islets, SMA^+ areas were found along most $CD34^+$ areas with the greatest density found in larger feeding arterioles at the periphery of the islets that extended inward for variable distances (Fig. 1A–C; SMA). Within the exocrine regions surrounding islets, SMA^+ cells were observed primarily at larger intralobular arterioles (not shown). Endothelial cells were detected using either CD34 or CD31 (Supplemental Fig. 1). The islet $CD34^+$ vessels were larger than the vessels in the surrounding exocrine regions in control donors and were widely scattered throughout the islet (Fig. 1A–C; CD34). Islet vessel diameter, density, and

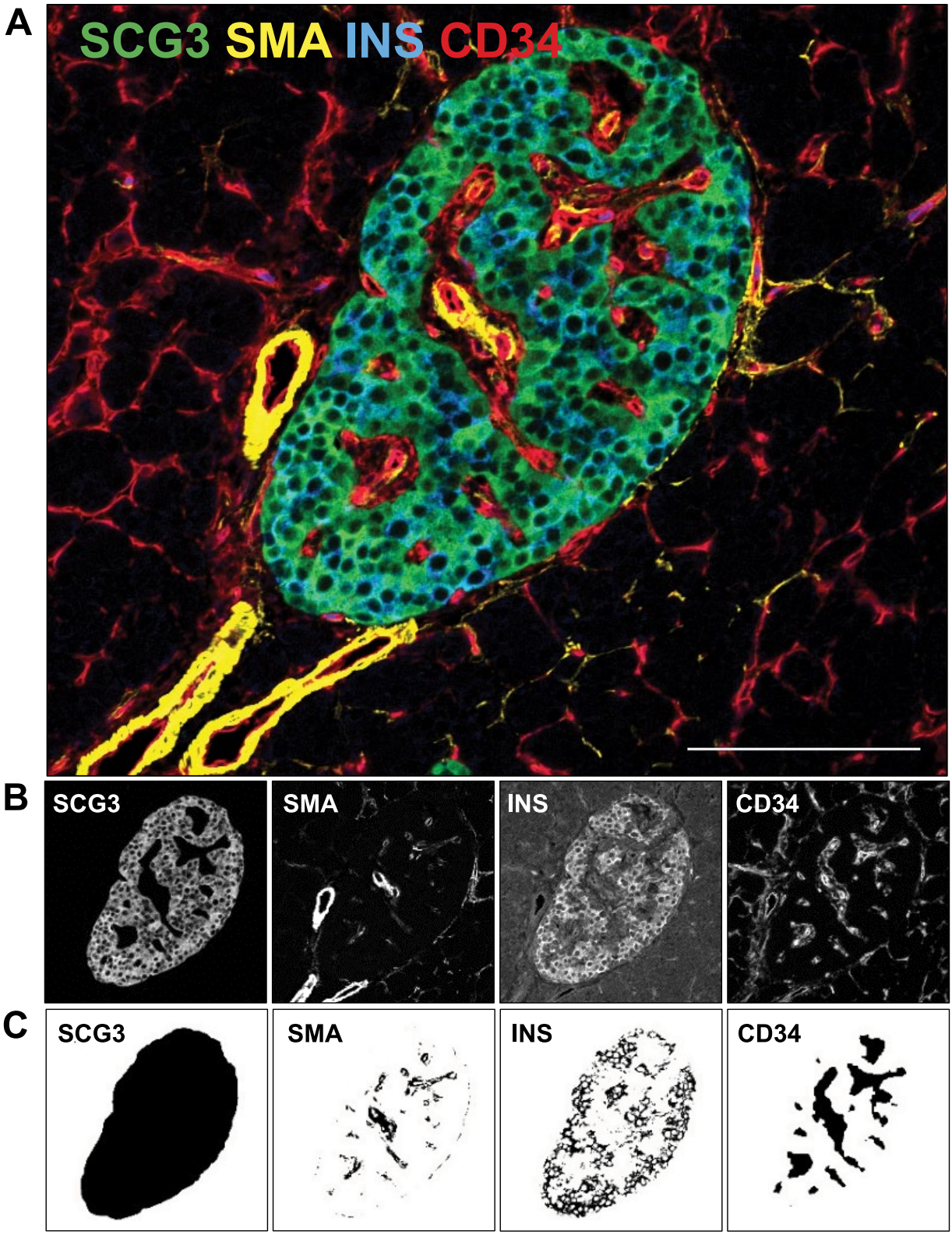

Figure 1. Image analysis procedure. A representative merged islet image is shown from a 20-year-old male African American donor without diabetes (A). Sections were stained for SCG3 (green, total islet), SMA (yellow, smooth muscle cells), insulin (blue, beta-cells), and CD34 (red, vessels) as described in section "Materials and Methods." Image analysis segmentation steps for each stain are shown before (B) and after (C) each processing step. Scale bar = 100 μm. Abbreviations: SCG3, secretogranin 3; SMA, smooth muscle actin; INS, insulin; CD34, vascular endothelium.

area were examined by donor age and diabetes status with no significant differences observed by donor age in either group (Supplemental Fig. 2). Larger islet vessels were observed in control donors (Fig. 2A and B) compared with islet vessels in diabetic islets (Fig. 2C and D). Quantification of islet vessel diameters showed a significant difference with smaller vessels in islets from type 1 diabetes donors compared with control islets (6.2 ± 0.8 µm vs. 7.6 ± 1.1 µm, respectively; Fig. 2E). Vessel density was significantly higher in type 1 diabetes donors compared with control donors (9.3 ± 0.2 × 10^{-4} vessels/µm^2 vs. 5.3 ± 0.6 ×10^4 vessels/µm^2 islet area, respectively; Fig. 2F). The overall mean islet vessel area (%) was similar between control donors and donors with type 1 diabetes (8.8 ± 2.0% vs. 9.7 ± 1.7%, respectively; p=0.22; Fig. 2G).

Further studies were conducted to validate initial findings with increased islet sample sizes based on a previous study indicating an optimal sample size of 30 islets.[31] Slides were reimaged and islets photographed and analyzed by a second observer (L.N.) using 5–35 islets per donor (n=3 control donors, n=4 donors with type 1 diabetes). Representative examples of vessel density are shown in Supplemental Fig. 3. Mean vessel density was similar from 5 to 35 islets examined from a single donor (Supplemental Fig. 3A). Mean islet vessel density and diameters were similar within donors examining 10 to 30 islets (Supplemental Fig. 3B and C).

Islet and Peri-islet Exocrine Vessel Parameters Were Different in Control Donors But Not in Donors With Type 1 Diabetes

Islet vessel diameters were significantly larger in islets compared with the peri-islet exocrine regions in control donors (7.6 ± 1.1 µm, 5.4 ± 0.2 µm, respectively; Fig. 3A). Islet vessel density was also significantly less than exocrine vessel density in control donors (5.3 ± 0.6 × 10^4 vessels/µm^2, 9.4 ± 1 × 10^4 vessels/µm^2, respectively; Fig. 3B). Significant differences were not observed between islet and exocrine vessel diameters for donors with type 1 diabetes (Fig. 3C and D) nor between controls and diabetic donor groups for exocrine vessel diameter, density, or area (Supplemental Fig. 4).

Diabetes Duration and Vessel Parameters

The potential relationship between vessel morphology and type 1 diabetes duration was also examined. Islet vessel diameter tended to decrease with increasing diabetes duration, though the correlation was not significant (p=0.052; Fig. 4A). Islet vessel density was significantly increased with longer diabetes duration (p<0.05; Fig. 4B).

Beta-cells in Islets From Donors With Type 1 Diabetes

Seven (41%) of the 17 donors with type 1 diabetes had variable numbers of residual beta-cells (INS$^+$) within islets (n=5–9 INS$^+$ islets/donor; Supplemental Table 1; Fig. 5). INS$^+$ islet vessel diameters were significantly larger (Fig. 5E) and had lower densities (Fig. 5F) compared with INS$^-$ islets within the same donor (n=5–6 INS$^-$ islets/donor). Significant correlations were not found between vessel diameter or vessel density versus islet insulin area (%; Supplemental Fig. 5). INS$^+$ islets from type 1 diabetes donors were further subtyped by insulitis status (CD3$^{+/-}$). INS$^+$CD3$^+$ islets were identified in 5 (71%) of the 7 diabetic donors (Supplemental Table 1). INS$^+$CD3$^+$ islets were infrequent (n=28 INS$^+$CD3$^+$ total from all 5 of the donors examined, n=4–7 islets/donor). The INS$^+$CD3$^+$ islet vessels were similar in diameter and density to those in INS$^+$CD3$^-$ islets (Supplemental Fig. 6).

Islet SMA Area Increased Significantly With Age

Islet SMA area (%) was variable between islets and was not significantly different between controls and donors with type 1 diabetes (Fig. 6A). Correlation analysis addressing an effect of age for the two donor groups did indicate a significant positive relationship for SMA area in control donors (r = 0.58, p=0.018) and donors with type 1 diabetes (r = 0.50, p=0.047; Fig. 3B).

Discussion

The study reported herein examined the morphology of pancreatic islet and exocrine vessels in organ donors with and without type 1 diabetes. While exocrine vessel diameters and density were similar between control and diabetic donors, significant abnormalities of the islet microvasculature morphology were observed in the patients with type 1 diabetes, with vessels having smaller diameters and higher density. These changes were significantly decreased with the presence of residual beta-cells. These findings suggest residual islet beta-cells may have a critical role in maintenance of islet vessel morphology, or alternatively, changes to the islet microvasculature may contribute to beta-cell loss.

The islet vessel diameters for control donors reported in this study using formalin-fixed paraffin sections (7.6 ± 1.1 µm) were higher than those determined

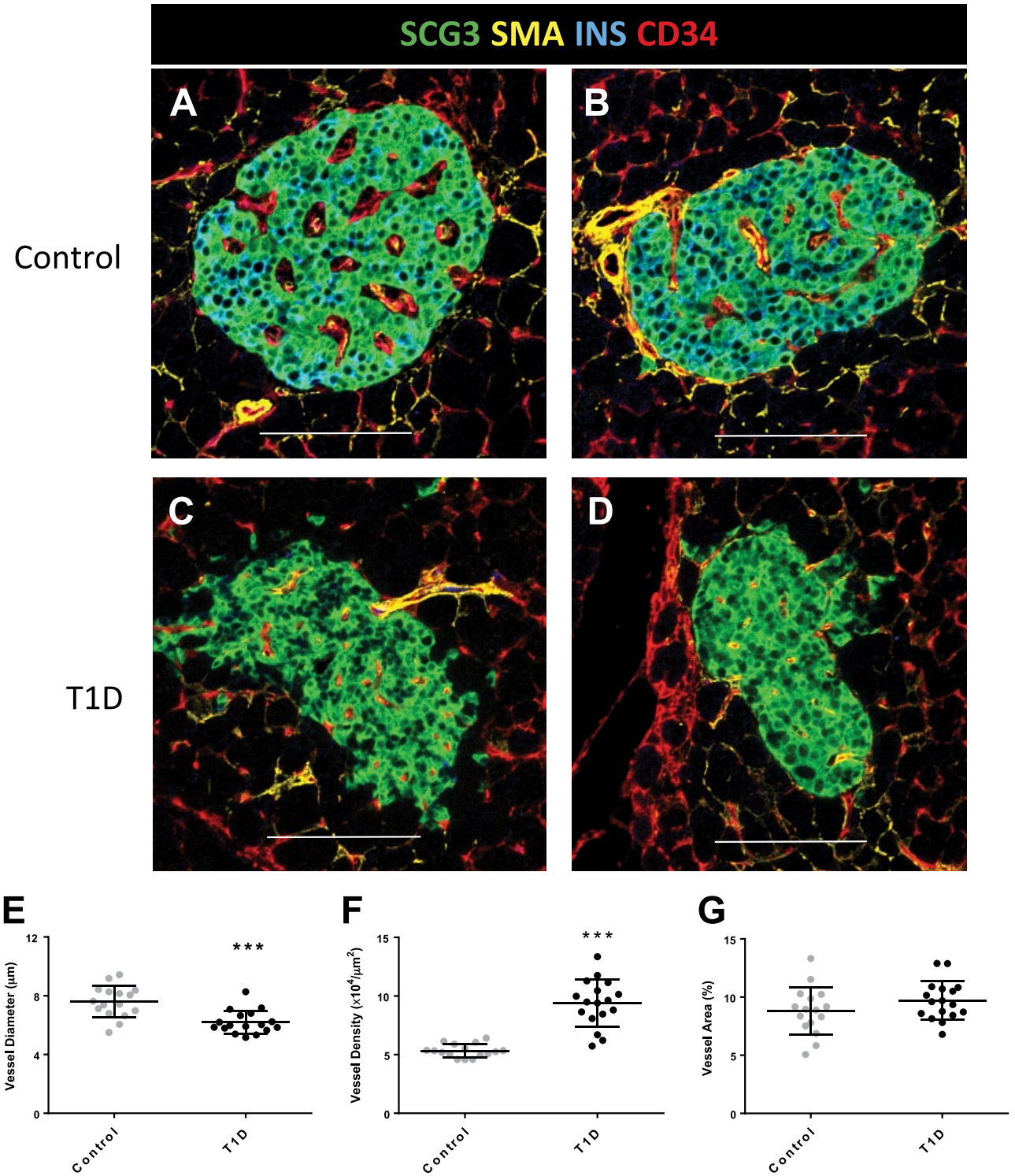

Figure 2. Islet vessels in control donors and donors with type 1 diabetes. Sections were stained and image analysis performed as described in section "Materials and Methods" for 16 control donors (*N*=10 islets/donor) and 17 donors with type 1 diabetes (*N*=7–20 islets/donor). Representative islets are shown from 2 control donors (A–B) and 2 diabetic donors (C–D). Islet vessel diameters from diabetic donors were significantly smaller than in control islets (E, ***$p<0.001$). Vessel density was significantly increased in diabetic islets compared with control islets (F, ***$p<0.001$). Islet vessel area (%) was not significantly different between donor groups (G). Scale bars = 100 µm. Abbreviations: SCG3, secretogranin 3; SMA, smooth muscle actin; INS, insulin; CD34, vascular endothelium.

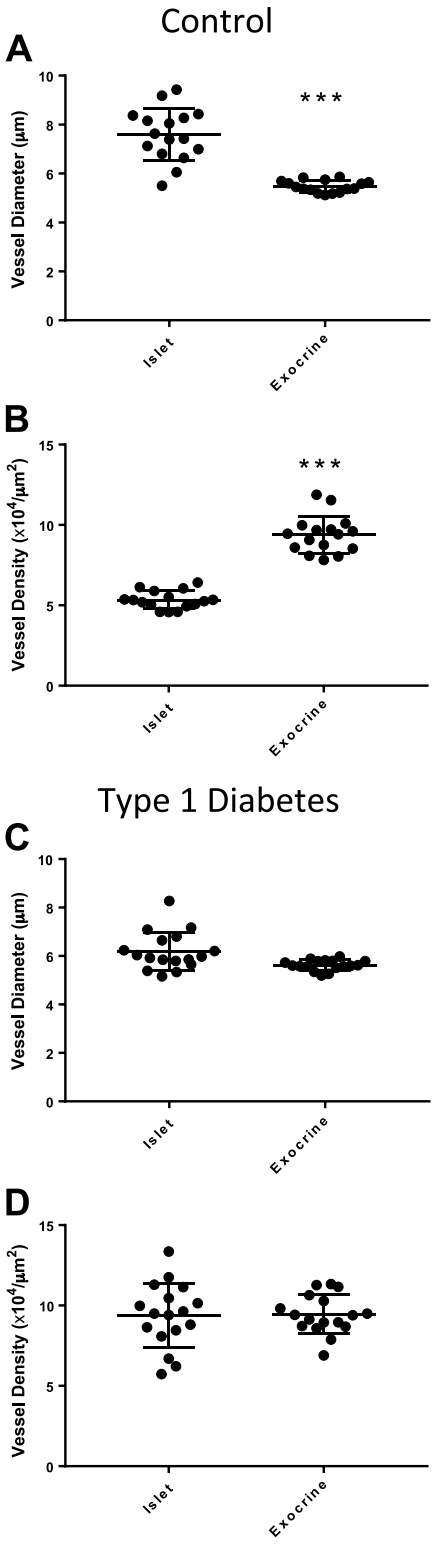

Figure 3. Islet and peri-islet exocrine vessels in control donors and donors with type 1 diabetes. Sections were stained and image analysis performed as described in section "Materials and Methods" for 16 control donors (N=10 islets/donor) and 17 donors with type 1 diabetes (N=7–20 islets/donor). Islet vessel diameters were significantly larger than peri-islet exocrine vessel diameters in control donors (A, ***p<0.001). Islet vessel density was significantly decreased compared with peri-islet exocrine vessel density in control islets (B, ***p<0.001). Islet and peri-islet exocrine vessel diameter and density were not significantly different in donors with type 1 diabetes (C–D, p>0.05). Scale bar = 100 µm.

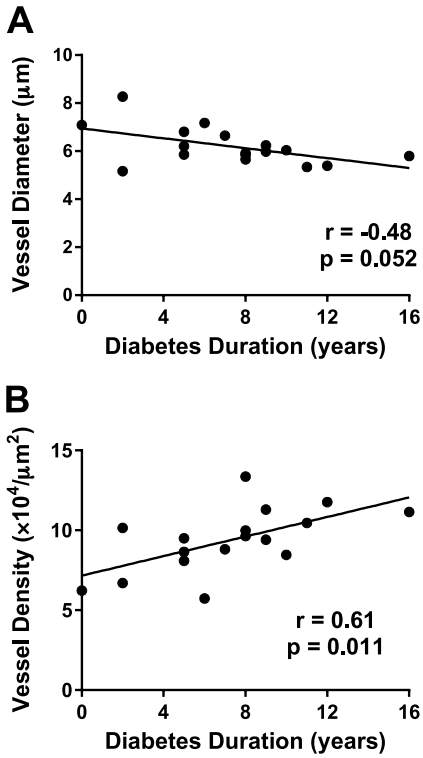

Figure 4. Diabetes duration and islet vessel diameter and density. Sections were stained and image analysis performed as described in section "Materials and Methods" for 17 donors with type 1 diabetes (N=7–20 islets/donor). Islet vessel diabetes (A) and density (B) were plotted as a function of diabetes duration (years), and correlation analysis was performed using Spearman's test with linear regression lines shown.

using fixed pancreas slices from patients undergoing partial pancreatectomy (5.1 ± 0.2 µm).[10] Methodological differences in sample processing (paraffin 4 µm vs. agarose-embedded 120 µm vibratome sections), staining (CD34 vs. lectin staining), and image analysis procedures could potentially explain the differences in absolute islet vessel diameter. Interestingly, mean islet vessel areas were remarkably similar between these two studies (9.7 ± 1.7% vs. 9.1 ± 0.5%) despite a large difference in control donor ages (18.0 ± 5.0, N=16, vs. 62.8 ± 5.0, N=4; mean ± SD). These data support our finding that donor age did not significantly impact

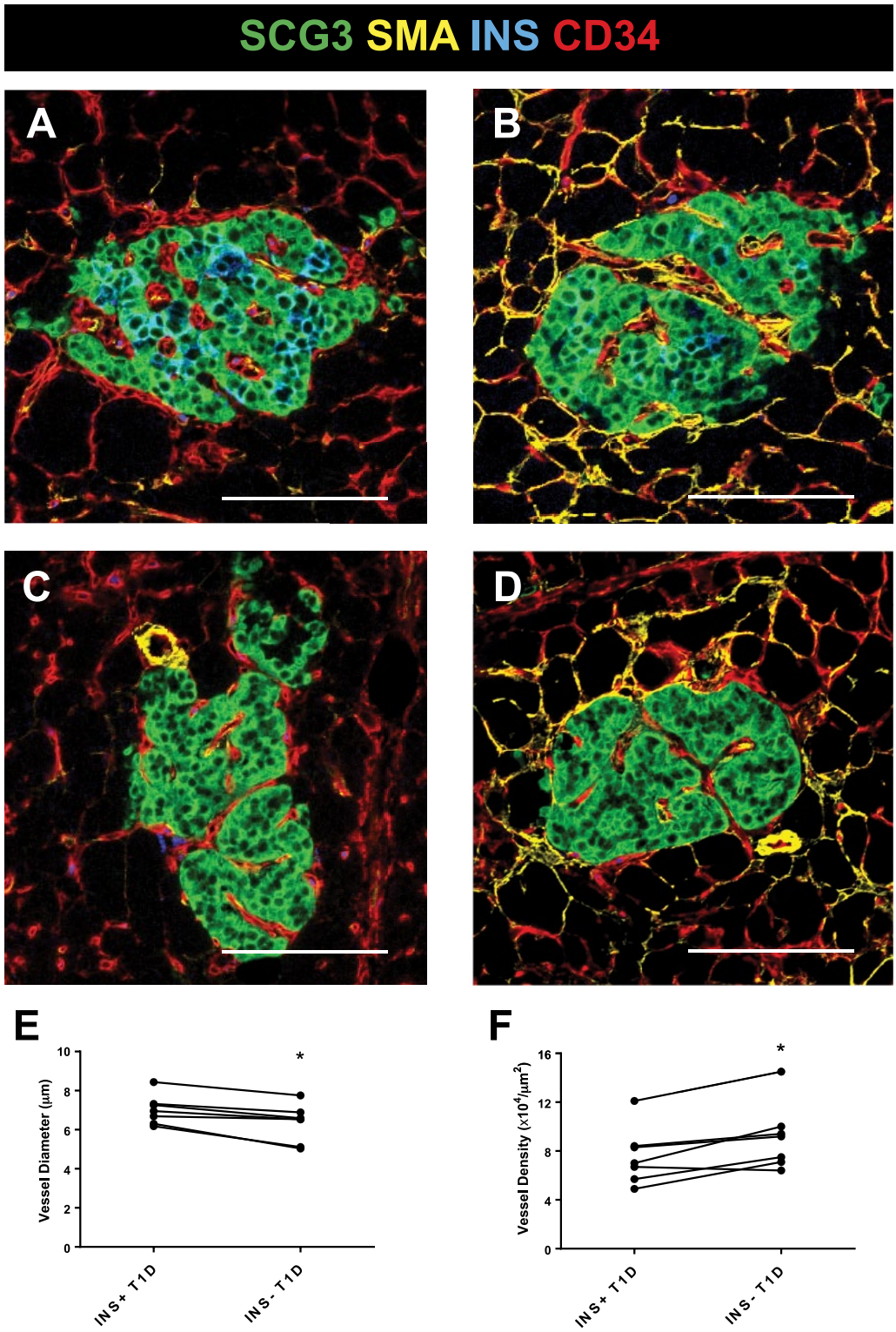

Figure 5. Beta-cells maintain vessel morphology in diabetic islets. Pancreas sections were stained, and image analysis performed as described in section "Materials and Methods" for 7 donors with type 1 diabetes (N=5–15 INS^+ islets/donor, N=4–6 INS^- islets/donor; Supplemental Table 1). Representative INS^+ (A–B) and INS^- (C–D) islets are shown from 2 diabetic donors. Significant differences were observed for vessel diameter (E, *p<0.05) and density (F, *p<0.05) by INS phenotype. Scale bars = 100 µm. Abbreviations: SCG3, secretogranin 3; SMA, smooth muscle actin; INS, insulin; CD34, vascular endothelium.

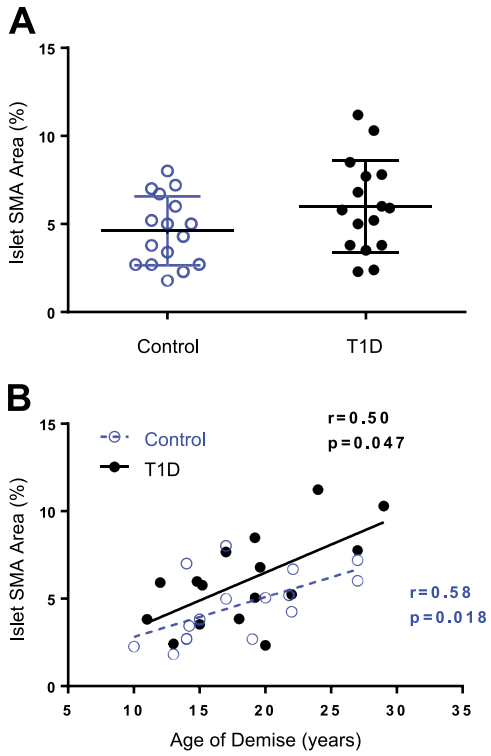

Figure 6. Islet SMA area and donor age. Pancreas sections were stained and image analysis performed as described in section "Materials and Methods" for 16 control donors (N=10 islets/donor) and 17 donors with type 1 diabetes (N=7–20 islets/donor). Islet SMA area (%) was plotted by donor group (A) and by age (years) for control donors (blue open circles, A) and diabetic donors (black closed circles, B). Islet SMA area was not significantly different between donor groups (Student's t-test, p=.151) but was significantly correlated with donor age in both groups (B). Linear regression line plotted with Spearman's r and p values, control slope = 0.23 ± 0.08, T1D slope = 0.32 ± 0.1. Abbreviations: SMA, smooth muscle actin; T1D, type 1 diabetes.

vessel parameters defined by $CD34^+$ staining. Cohrs et al. also found that islet size did not impact islet vessel area in either mouse or human pancreas.[10]

The islet microvasculature in patients with type 2 diabetes has been studied by two groups, and one showed an increase in vessel density compared with control donors.[20] Our results obtained from patients with type 1 diabetes also showed a significant increase in vessel density compared with control donors, suggesting a potential link between dysglycemia and vessel density. Due to the decrease in vessel diameter, islet vessel areas (%) were similar between control and type 1 diabetes islets. These data contrast with a recent study showing increased islet vessel area in donors with type 2 diabetes.[23] Islets with increased vessel numbers and smaller diameters could imply increased vessel tortuosity following beta-cell loss. The physiological consequences of these morphological changes in islet vasculature remodeling with beta-cell loss are unknown but could impact functions of the remaining neuroendocrine cells, including alpha-cells.

The interdependence between beta-cells and vascular endothelial cells is well-described (reviewed in[9,14]). The development, maintenance, and proper function of the islet vasculature is dependent upon vascular endothelial growth factor (VEGF)-A signaling, which is largely produced by beta-cells.[32] Insulin is also a known vasodilator via activation of endothelial nitric oxide (NO) synthase after insulin receptor binding.[33] A major finding of our study showed that islets from diabetic donors with residual beta-cells had vessel diameters and densities more similar to control islets. Such findings suggest a normal islet microenvironment may be more conducive to beta-cell longevity.

Species differences in islet vessel SMA area are known with 3-fold greater SMA area in humans compared with rodents.[21] While not different between donor groups, the SMA area did significantly increase with age in both control and diabetic donors. Sympathetic innervation of islet smooth muscle cells has been proposed to be the primary mechanism by which the sympathetic arm of the peripheral nervous system inhibits insulin secretion in humans, that is, vasoconstriction and reduced blood flow rate.[21] In addition to the loss of vasodilatory mediators released by beta-cells, smaller vessels in islets of donors with type 1 diabetes could impact responses to autonomic or sensory neurotransmitters by alpha- and delta-cells (glucagon, somatostatin, respectively). Such findings could also have importance in islet transplantation as a negative correlation was observed between donor age and vascular density when human islets were transplanted under the mouse kidney capsule.[34]

In summation, several key alterations of the human islet microvasculature were identified in patients with type 1 diabetes. Residual beta-cells in islets from patients with type 1 diabetes were associated with islet vessels having greater diameters and decreased density comparable to islets from control donors. These findings confirm a critical relationship between beta-cells and maintenance of the islet microvasculature. Furthermore, the physiological significance of higher SMA area in older donors may underscore poorer islet transplantation survival from aged donors. Future studies are planned to examine potential mechanisms underlying these morphological findings including expression of VEGF or relevant VEGF receptors and neural input to the vascular smooth muscle cells in islets from patients with type 1 diabetes.

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Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

All authors have contributed to this article as follows: JSC performed the IHC, designed the image analysis, analyzed data, and wrote the manuscript; EAB and DAF performed IHC and reviewed the manuscript; LHN analyzed data and reviewed the manuscript; MAA reviewed the manuscript; MC-T designed the experiments, analyzed data, and wrote the manuscript; and all authors have read and approved the manuscript as submitted. MC-T is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of these data and the accuracy of the data analysis.

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ORCID iDs

MA Atkinson https://orcid.org/0000-0001-8489-4782
M Campbell-Thompson https://orcid.org/0000-0001-6878-1235

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