Pancreas Volume Declines During the First Year After Diagnosis of Type 1 Diabetes and Exhibits Altered Diffusion at Disease Onset

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Diabetes Care 2019;42:248–257 | https://doi.org/10.2337/dc18-1507

OBJECTIVE

This study investigated the temporal dynamics of pancreas volume and microstructure in children and adolescents with recent-onset type 1 diabetes (T1D) and individuals without diabetes, including a subset expressing autoantibodies associated with the early stages of T1D.

RESEARCH DESIGN AND METHODS

MRI was performed in individuals with recent-onset stage 3 T1D (n = 51; median age 13 years) within 100 days after diagnosis (mean 67 days), 6 months, and 1 year postdiagnosis. Longitudinal MRI measurements were also made in similarly aged control participants (n = 57) and in autoantibody-positive individuals without diabetes (n = 20). The MRI protocol consisted of anatomical imaging to determine pancreas volume and quantitative MRI protocols interrogating tissue microstructure and composition.

RESULTS

Within 100 days of diabetes onset, individuals with T1D had a smaller pancreas (median volume 28.6 mL) than control participants (median volume 48.4 mL; P < 0.001), including when normalized by individual weight (P < 0.001). Longitudinal measurements of pancreas volume increased in control participants over the year, consistent with adolescent growth, but pancreas volume declined over the first year after T1D diagnosis (P < 0.001). In multiple autoantibody–positive individuals, the pancreas volume was significantly larger than that of the T1D cohort (P = 0.017) but smaller than that of the control cohort (P = 0.04). Diffusion-weighted MRI showed that individuals with recent-onset T1D had a higher apparent diffusion coefficient (P = 0.012), suggesting a loss of cellular structural integrity, with heterogeneous pancreatic distribution.

CONCLUSIONS

These results indicate that pancreas volume is decreased in stages 1, 2, and 3 of T1D and decreases during the first year after diabetes onset and that this loss of pancreatic volume is accompanied by microstructural changes.

Reduced pancreas size has been noted in individuals with long-standing type 1 diabetes (T1D) (1–3). Because pancreatic islets comprise only 1–2% of the pancreatic mass, the reason for the smaller pancreas in T1D is not known, but changes in the exocrine pancreas are implicated. More recently, studies in adults with recent-onset

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Received 13 July 2018 and accepted 15 November 2018

Clinical trial reg. no. NCT03585153, clinicaltrials.gov

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-1507/-/DC1.

T1D have demonstrated reduced pancreatic volume within the first year after diagnosis (4,5). The development of T1D is currently thought to progress from autoimmunity as reflected by islet autoantibodies (stage 1), through dysglycemia (stage 2), and ultimately clinical manifestation of diabetes (stage 3) (6). Surprisingly, one autopsy study found that pancreas weight was less in individuals without diabetes (n = 8) expressing autoantibodies that portend the development of T1D (7). These studies raise the possibility that pancreas volume declines early in the course of T1D progression or that individuals who develop T1D may have a smaller pancreas prior to the onset of stage 3 T1D. A number of cross-sectional studies have examined pancreas volume as a function of T1D duration with conflicting results (1,8–11). These cross-sectional studies are hampered by the fact that pancreas volume is a function of age (12,13) and that large interindividual differences exist in the volume of the pancreas (14). Longitudinal studies of pancreas volume in children or adolescents with T1D have not been performed to determine how the pancreas changes during normal adolescence or in the early stages of T1D.

MRI has a number of advantages for assessing the pancreas. Unlike computed tomography (CT), MRI does not use ionizing radiation, enabling longitudinal MRI measurements to be made in children and adolescents at frequent intervals with no known risk. Additionally, a meta-analysis comparing studies using MRI and CT to interrogate the pancreas in diabetes demonstrated a larger difference in pancreas volume when using MRI as well as lower study heterogeneity in studies using MRI than those performing CT (9). In addition to excellent soft tissue contrast, MRI can quantitatively assess other aspects of pancreas structure and composition. These quantitative MRI techniques include diffusion-weighted MRI (DW-MRI), which measures the diffusion of water in tissue and has been used in studies of both pancreatic cancer (15) and pancreatitis (16). Another quantitative MRI technique maps the transverse relaxation time (T2), which may reflect tissue edema and has previously been shown to be elevated in the NOD mouse model of T1D (17). A third quantitative technique is magnetization transfer (MT) MRI, which measures the size of the macromolecular pool in tissue and has been shown to correlate with fibrosis (17). A final MRI technique of interest is a ratio of the pancreas to the spleen signal on a T1-weighted image, which has previously been shown to reflect exocrine dysfunction in chronic pancreatitis (18). Importantly, these quantitative MRI techniques have not been applied to study the pancreas in T1D. Furthermore, these techniques create maps of the entire pancreas and can thus identify regions of the pancreas with altered characteristics, such as lobular insults thought to underlie T1D (19).

To define the dynamics of pancreas volume and assess alterations in microstructure in the early clinical stages of T1D, we performed longitudinal, quantitative MRI in individuals with recent-onset T1D, autoantibody-positive individuals with T1D identified in the Type 1 Diabetes TrialNet Pathway to Prevention study, and similarly aged control participants.

RESEARCH DESIGN AND METHODS

Study Participants

Participants with recent-onset T1D were recruited from a weekly recent-onset T1D educational class in the Eskind Pediatric Diabetes Clinic at Vanderbilt University Medical Center. Participants who were recently diagnosed with T1D ($<$100 days since diagnosis) were offered participation in the study. The clinical diagnosis of T1D was established by the participants' endocrinologist based on the American Diabetes Association criteria for T1D. Healthy control participants consisted of similarly aged volunteers with no known pancreas pathology. T1D autoantibody–positive participants were relatives without diabetes of patients with T1D who were recruited from the participant pool of the Type 1 Diabetes TrialNet Clinical Center at Vanderbilt through the TrialNet Natural History Study (subsequently renamed the TrialNet Pathway to Prevention Study) (20). This trial screened relatives without diabetes of probands with T1D, including first-degree relatives (sibling, parent, or child) who were aged 3 through 45 years, as well as second- or third-degree relatives (niece, nephew, aunt, uncle, or cousin) who were aged 3 through 20 years for antibodies to insulin, GAD, and insulinoma-associated antigen-2. Islet cell and zinc transporter 8 autoantibodies were measured if at least one other antibody tested positive. All autoantibody-positive participants were positive for more than one autoantibody prior to enrollment in this study. All protocols were approved by the Vanderbilt Institutional Review Board. The study was registered at ClinicalTrials.gov under unique identifier NCT03585153.

A number of clinical metrics were captured from the Vanderbilt electronic medical record for each participant with T1D in this study: basic demographic data, family history of T1D, medication use, and presence of comorbidities. Participants with T1D were surveyed for presence and severity of diabetic ketoacidosis (defined as pH $<$7.3 or bicarbonate $<$15 mEq/L) or pancreatitis. Participants with T1D were metabolically stable prior to their first MRI, with stable insulin dosage and weight. T1D management included multiple daily insulin injections including both short- and long-acting insulin analogs or continuous subcutaneous insulin infusions. At each imaging session, participant insulin use, A1C, autoantibody status, C-peptide, and blood glucose were recorded from their clinical record, where available.

MRI

MRI was performed using a Philips 3T Achieva MR scanner and a 16-channel torso coil (Philips Healthcare, Best, the Netherlands). All imaging was performed in the axial plane using fat-suppression techniques. Care was taken to capture the entire pancreas volume during each imaging sequence. Individuals were not fasted prior to imaging. For anatomical imaging, two imaging sequences were performed. The first anatomical sequence was a T2-weighted fast-spin echo sequence with spatial resolution of 1.5 $\times$ 1.5 $\times$ 5 mm and repetition time (TR) and echo time (TE) of 840 ms and 70 ms, respectively. The scan was completed in two breath holds in a total imaging time of 25 s. The second anatomical image was a T1-weighted ultrafast gradient echo sequence with spatial resolution of 1.5 $\times$ 1.5 $\times$ 4 mm and TR/TE of 3.4/1.68 ms. This scan was completed in a single 17-s breath hold.

The pancreas was outlined on contiguous slices of the digital T2-weighted images by an experienced radiologist (M.H.) blinded to the diabetes status of each participant. Pancreas outlining was performed using the Medical Image

Processing, Analysis, and Visualization application developed by the National Institutes of Health (NIH) (https://mipav.cit.nih.gov/). The T1-weighted image was consulted for slices on which the pancreas border was unclear on the T2-weighted image and used to guide delineation of the pancreas border. Pancreas volume was calculated by multiplying the sum of the pancreas area on each slice by the slice thickness (21). The pancreas volume outlined by the radiologist was regridded to the image resolution of the quantitative techniques (described below) to delineate the pancreas volume on each parametric map. Each MRI parameter was averaged throughout the entire pancreas volume, except for the pancreas-to-spleen T1 ratio, which was quantified using a region of interest as described below. Images were inspected to ensure that patient motion between image acquisitions was minimal.

DW-MRI

A single-shot echo planar imaging sequence was used for diffusion-weighted imaging, similarly to previously described methods (22). Spatial resolution was 2.4 × 2.4 × 5 mm, and TR/TE was 1,700/74 ms. Total image acquisition time was 6 min 48 s. Diffusion-weighting was encoded in three orthogonal directions (*x, y*, and *z*) with *b* values of 0, 25, 50, 75, 100, 200, and 800 s/mm^2. Apparent diffusion coefficient (ADC) maps were calculated from the images taken with *b* = 200 and 800 s/mm^2. ADC values were computed for each voxel by fitting the signal intensities to Eq. 1:

$$SI_{(b\,=\,800)} = SI_{(B\,=\,200)} \times e^{-ADC \times (800-200)} \quad (1)$$

where $SI_{(b\,=\,800)}$ and $SI_{(b\,=\,200)}$ are the signal intensities at *b* values of 800 and 200, respectively.

T2 Map

T2-weighted images were acquired using a multiple-echo turbo spin-echo pulse sequence at six echo times (12, 30, 48, 65, 83, and 100 ms), using well-established techniques (23). Spatial resolution was 2.4 × 2.4 × 5 mm, and TR was 3,400 ms. Image acquisition time was 3 min 44 s. Quantitative maps of the transverse relaxation time, T2, were calculated on a voxel-by-voxel basis by fitting the multiecho data to the mono-exponential function shown in Eq. 2:

$$SI(TE) = S_0 \times e^{-\frac{TE}{T2}} \quad (2)$$

where SI(TE) is the signal intensity at each echo time, S_0 is the proton density, and TE is the echo time.

MT MRI

A three-dimensional gradient echo sequence was performed with spatial resolution of 2.4 × 2.4 × 5 mm and TR/TE of 45/2.9 ms. Two images were performed during the course of separate breath holds, each identical save for the inclusion of an MT saturation pulse of duration 20 ms and flip angle 800° set 1,500 Hz off of the water resonant frequency for one scan. Total acquisition time for both images was 29 s. MT ratio (MTR) maps were calculated for each voxel using Eq. 3:

$$MTR = \frac{SI_{MT\_off} - SI_{MT\_on}}{SI_{MT\_off}} \quad (3)$$

where SI_{MT_on} and SI_{MT_off} are the signal intensities with and without the MT saturation pulse, respectively (24).

Pancreas-to-Spleen T1 Ratio

A single slice of the T1-weighted ultrafast gradient echo sequence was chosen containing both the tail of the pancreas and spleen. A region of interest of the same size (~2 cm^2) was drawn in relatively homogeneous regions of both the pancreas and spleen. The average pancreas signal throughout the region of interest was divided by average spleen signal throughout the region of interest to calculate the pancreas-to-spleen ratio, similarly to methods previously described (18).

Mixed-Meal Tolerance Testing/Oral Glucose Tolerance Testing

A mixed-meal tolerance test (MMTT) was administered to assess participants' insulin secretion capacity for a subset of the T1D and control participants who opted in to this part of the study. Briefly, each participant was instructed to eat a high-carbohydrate diet (≥150 g) for 3 days prior to the test and fast beginning at 10:00 P.M. the night before the MMTT. The fasted participant was administered an oral challenge (Boost HP; 6 mL/kg body weight), and serum glucose and C-peptide measurements were performed at −10, 0, 15, 30, 60, 90, and 120 min after oral challenge. In the autoantibody-positive cohort, oral glucose tolerance testing (OGTT) was performed in a subset of participants in accordance with their participation in the Pathway to Prevention study protocol. The primary difference for the OGTT was that participants were administered oral glucose as a challenge at 1.75 g/kg body weight. C-peptide production was estimated from MMTT and OGTT by determining the area under the curve of C-peptide production for 2 h following oral challenge (25).

Data Management and Statistical Analysis

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Vanderbilt University Medical Center (26). REDCap is a secure, web-based application designed to support data capture for clinical research studies, providing: *1*) an intuitive interface for validated data entry; *2*) audit trails for tracking data manipulation and export procedures; *3*) automated export procedures for seamless data downloads to common statistical packages; and *4*) procedures for importing data from external sources.

Statistical analysis was performed using R (version 3.3.2). All tests were two-sided with a *P* value of <0.05 considered statistically significant. For unpaired continuous variables, Kruskal-Wallis testing was performed to compare continuous variables with more than two groups, and the Wilcoxon rank-sum test with continuity correction was used to compare two groups (including post hoc testing). For paired continuous variables, the Wilcoxon matched-pairs signed-rank test was used. The correlation between variables was assessed using Spearman rank-order correlation. Multivariable linear regressions with age, sex, and BMI as covariates were applied to estimate the adjusted difference between groups at the first MRI time point. Longitudinal data were analyzed using linear mixed-effects models with random intercept and autoregressive correlation incorporating age at first MRI, sex, time since first MRI, whether participant was in the control or T1D cohort, and the interaction between group identity and time. The repeatability of pancreas volume index measurements was assessed using the interclass correlation coefficient (ICC) of longitudinal

measurements in control participants between the first and second MRI.

The Kolmogorov-Smirnov test was used to compare the distribution of voxel values in the pancreas between the control and T1D groups. In addition, the difference in the median, lower quartile, and upper quartile between the control and T1D groups was calculated from measured voxel values. *P* values were obtained by comparing these observed differences with their null distributions generated from permutations of the data.

RESULTS

Pancreas Volume Is Smaller at T1D Onset and Continues to Decline Over Time

To investigate the dynamics of pancreas volume over the first year after diagnosis with T1D, we performed longitudinal MRI in individuals with recent-onset T1D (mean time since diagnosis 67 days) and similarly aged control participants. Most of the participants were children or adolescents (Table 1). A schematic of the study design is shown in Fig. 1*A*. Of the 57 control participants, 51 participants with T1D, and 20 autoantibody-positive participants who were scanned at the first MRI, 53 control participants (93%), 47 participants with T1D (92%), and 14 autoantibody-positive participants (70%) completed the second MRI, and 43 control participants (75%), 37 participants with T1D (73%), and 8 autoantibody-positive participants (40%) completed the third MRI. The study is ongoing and collecting longitudinal data on these cohorts; second and third MRIs are scheduled for current participants that have not reached this time point.

An example image of the pancreas of a control participant and participant with recent-onset T1D is shown in Fig. 1*B* and *C*, respectively, with the pancreas outlined in white. To account for normal adolescent growth, we compared pancreas volume and weight in control study participants. In the control cohort, pancreas volume correlated with participant weight (Fig. 1*D*) ($R = 0.76$; $P < 0.001$). Thus, to account for changes in pancreatic volume during normal adolescent growth, pancreas volume was normalized by participant weight, as previously described (5,7,11), yielding what has been termed the pancreas volume index. At the first study time point, the pancreas volume index was smaller in participants with recent-onset T1D than control participants (Fig. 1*E*) (median 0.929 mL/kg in control participants and 0.600 mL/kg in participants with T1D; $P < 0.001$). Pancreas volume index was smaller in participants with T1D when adjusted for age, sex, and BMI (mean difference −0.286 mL/kg [95% CI −0.360 to −0.212]; $P < 0.0001$ from linear regression). Additionally, unnormalized pancreas volume was smaller in participants with recent-onset T1D than in control participants (Supplementary Fig. 1*A*) ($P < 0.001$), as well as when adjusted for age, sex, and BMI (mean difference −13.9 mL [95% CI −18.2 to −9.7]; $P < 0.0001$).

Longitudinal MRI of the pancreas at 6 and 12 months after diagnosis with T1D revealed a decline in pancreas volume index in recent-onset T1D compared with the control cohort in whom the pancreas volume index was stable (Fig. 1*F*) (estimated slope from linear mixed-effects model for T1D, −0.0084 mL/kg/month [95% CI −0.0125 to −0.0043]; $P < 0.0001$; for control participants, −0.0012 [95% CI −0.0048 to 0.0025], $P = 0.53$; and difference in slopes between control and T1D cohorts, $P < 0.01$). Similar to pancreas volume index, pancreas volume displayed different dynamics between the control and T1D cohorts (Supplementary Fig. 1*B*). To account for interparticipant differences in pancreas volume at baseline, pancreas volume was subsequently normalized to the value at the first study point. Changes in pancreas volume relative to the measurement at the first time point, shown in Fig. 1*G*, demonstrate pancreas growth corresponding with normal adolescent growth in control participants (average rate of increase 0.7% per month with 95% CI 0.2–1.1% estimated from linear mixed-effects model; $P < 0.01$). However, participants with T1D demonstrated a decline in relative pancreas volume (average rate of decrease 0.6% per month [95% CI −0.1% to −1.1%], $P < 0.05$, and $P < 0.001$ for difference from control). Pancreas volume index was similarly normalized to the measurement at the first MRI. This relative pancreas volume index tended to increase or to be stable in control participants at an average rate of 0.04% (95% CI −0.3% to 0.4%; $P = 0.85$) per month but declined over the first year of T1D at an average rate of −1.2% (95% CI −0.7 to −1.7%, $P < 0.0001$) per month (Supplementary Fig. 1*C*) ($P < 0.001$ for slope difference between control and T1D cohorts from linear mixed-effects model). Repeatability of pancreas volume index was assessed using the ICC of longitudinal measurements in control participants. The ICC between the first and second MRI was 0.823 (95% CI 0.713–0.894), indicating excellent repeatability (27). Collectively, these results indicate not only that pancreas volume is smaller within weeks of diagnosis with T1D, but also that the pancreas volume declines in the first year after diagnosis with T1D.

As part of the recruitment process, we also enrolled normal control participants, some of whom were siblings of participants with T1D. This cohort of 27 control participants who were siblings of participants with recent-onset T1D had a pancreas volume similar to that of the other members of the control cohort ($P = 0.1$). Of the 27 sibling dyads in which one sibling had T1D, the sibling with T1D had a smaller pancreas volume index in 22 of these pairs (Supplementary Fig. 1*D*) ($P < 0.0001$). Sixteen of the 27 control participants who have siblings with T1D had autoantibody testing that was negative, whereas the other 11 control participants who had siblings with T1D did not undergo autoantibody testing. The number of first-degree relatives with T1D for each cohort are shown in Table 1. Note that this includes participants with multiple first-degree relatives with T1D.

Of the 51 participants with recent-onset T1D in the study, 22 had diabetic ketoacidosis at T1D onset. Pancreas volume index, measured when individuals were metabolically stable, was similar to that of participants with T1D who did not have diabetic ketoacidosis (Supplementary Fig. 1*E*) ($P = 0.50$). Furthermore, pancreatic volume index did not demonstrate correlation with either sex or race ($P = 0.5$ and $P = 0.1$, respectively).

Pancreas Volume Does Not Correlate With Common Clinical Metrics of T1D

To determine whether the decline in pancreas volume seen in T1D was associated with disease severity, pancreas volume index was compared with measures of glycemic control and insulin production. MMTT performed at the first

Table 1—Demographic characteristics of the study population

	Control (n = 57)	T1D (n = 51)	Autoantibody-positive (n = 20)	P value
Age	15.9 ± 6.7	13.4 ± 4.1	16.2 ± 5.8	0.055
BMI	21.4 ± 4.4	20.5 ± 4.1	22.9 ± 5.0	0.083
Sex				0.470
Female	42 (24)	51 (26)	55 (11)	
Male	58 (33)	49 (25)	44 (9)	
Race				0.114
Asian	5 (3)	0 (0)	0 (0)	
Black	16 (9)	6 (3)	5 (1)	
White	79 (45)	94 (48)	95 (19)	
Autoantibodies at time of first MRI				
Unknown	68 (39)	12 (6)	0 (0)	
0	32 (18)	2 (1)	0 (0)	
1	0 (0)	43 (22)	15 (3)	
2	0 (0)	31 (16)	40 (8)	
3	0 (0)	10 (5)	5 (1)	
4	0 (0)	2 (1)	25 (5)	
5	0 (0)	—	15 (3)	
A1C (%)				
Diagnosis	—	11.9 ± 2.3 (49)	—	
3 months postdiagnosis	—	6.8 ± 1.1 (48)	—	
6 months postdiagnosis	—	7.1 ± 1.5 (45)	—	
12 months postdiagnosis	—	7.7 ± 1.5 (40)	—	
First-degree relatives with T1D				
Father	2 (1)	4 (2)	10 (2)	
Mother	4 (2)	4 (2)	10 (2)	
Brother	21 (12)	2 (1)	20 (4)	
Sister	26 (15)	4 (2)	50 (10)	

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as percentages. For all variables, numbers in parentheses indicate the numbers of observations. The symbol "—" indicates the measurement was not performed.

time point demonstrated little evidence of the relationship between C-peptide production and pancreas volume index in either participants with T1D (n = 8; P = 0.5) or control participants (n = 14; P = 0.5) (Supplementary Fig. 1*F*). The C-peptide measurements with corresponding blood glucose and pancreas volume indices are shown in Supplementary Table 1. Pancreas volume index did not correlate with A1C (Supplementary Fig. 2*A*) at the first MRI time point (P = 0.34), at the second MRI performed 6 months after diagnosis (P = 0.75), or at the third MRI performed 1 year after diagnosis (P = 0.83). Similarly, pancreas volume index did not show evidence of correlation with C-peptide measured at disease onset (P = 0.28; R = 0.26) (Supplementary Fig. 2*B*). The lack of evidence of correlation between measures of pancreas volume index and insulin production or glycemic control suggests that pancreas volume may mark a process of T1D not captured by currently available assessments.

Pancreas Volume in Autoantibody-Positive Participants Is Intermediate Between Participants With T1D and Control Participants

To investigate whether declines in pancreas volume precede diagnosis with T1D, we performed longitudinal MRI in participants expressing autoantibodies associated with T1D. At the first study time point, the median pancreas volume index in the autoantibody-positive cohort with two or more autoantibodies present at the time of the MRI (n = 17) was larger than that in the T1D cohort ($P < 0.05$) but smaller than that in the control cohort ($P < 0.05$) (Fig. 2*A*). Pancreas volume displayed a range of distribution of pancreas volume similar to that in control individuals (Fig. 2*B*). The autoantibody-positive cohort included three participants who were positive for two or more autoantibodies at one point during their monitoring but had reverted to a single autoantibody at the time of the first MRI. The pancreas volume index of these three single autoantibody-positive participants is included in Supplementary Fig. 3*A* and *B*, demonstrating that pancreas volume index and pancreas volume in single autoantibody individuals may be similar to that in control participants. Acknowledging this is a small number of participants, the pancreas volume index in autoantibody-positive participants over time (Fig. 2*C*) displayed no clear trend, nor did measurements of pancreas volume over time (Supplementary Fig. 3*C*).

Similar to those for the control and T1D participants, pancreas volume and pancreas volume index were normalized by the baseline measurements to account for individual differences in these measurements at study entry. This relative pancreas volume displayed no clear average trend in autoantibody-positive participants throughout the study period (Fig. 2*D*), nor did the relative pancreas volume index (Supplementary Fig. 3*D*). However, two participants with multiple autoantibodies had dysglycemia during the course of the study (defined as stage 2 T1D [6]). Longitudinal measurements of pancreas volume index are shown for these two participants in Fig. 2*E*, along with lines indicating the average and 95% CI of the control and autoantibody-positive cohorts. Note that both participants who developed stage 2 T1D had a smaller pancreas volume index compared with the autoantibody-positive cohort average. One of these participants subsequently reverted to stage 1 T1D with normal glucose tolerance testing, whereas the other participant progressed to stage 3 T1D. The pancreas volume index was higher in the participant who experienced disease remission and displayed an initial decline in pancreas volume with subsequent increase. The pancreas volume index in the participant who progressed to T1D was lower than that in the participant who experienced remission. Pancreas volume index in autoantibody-positive participants did not show evidence of correlation with C-peptide production as assessed by OGTT (Fig. 2*F*). Collectively, these results indicate that autoantibody-positive individuals with lower pancreas volume may be at greater risk for disease progression.

Quantitative MRI of the Pancreas at Onset of T1D Suggests Focal Altered Diffusion

To assay changes in pancreas structure or content accompanying T1D, quantitative

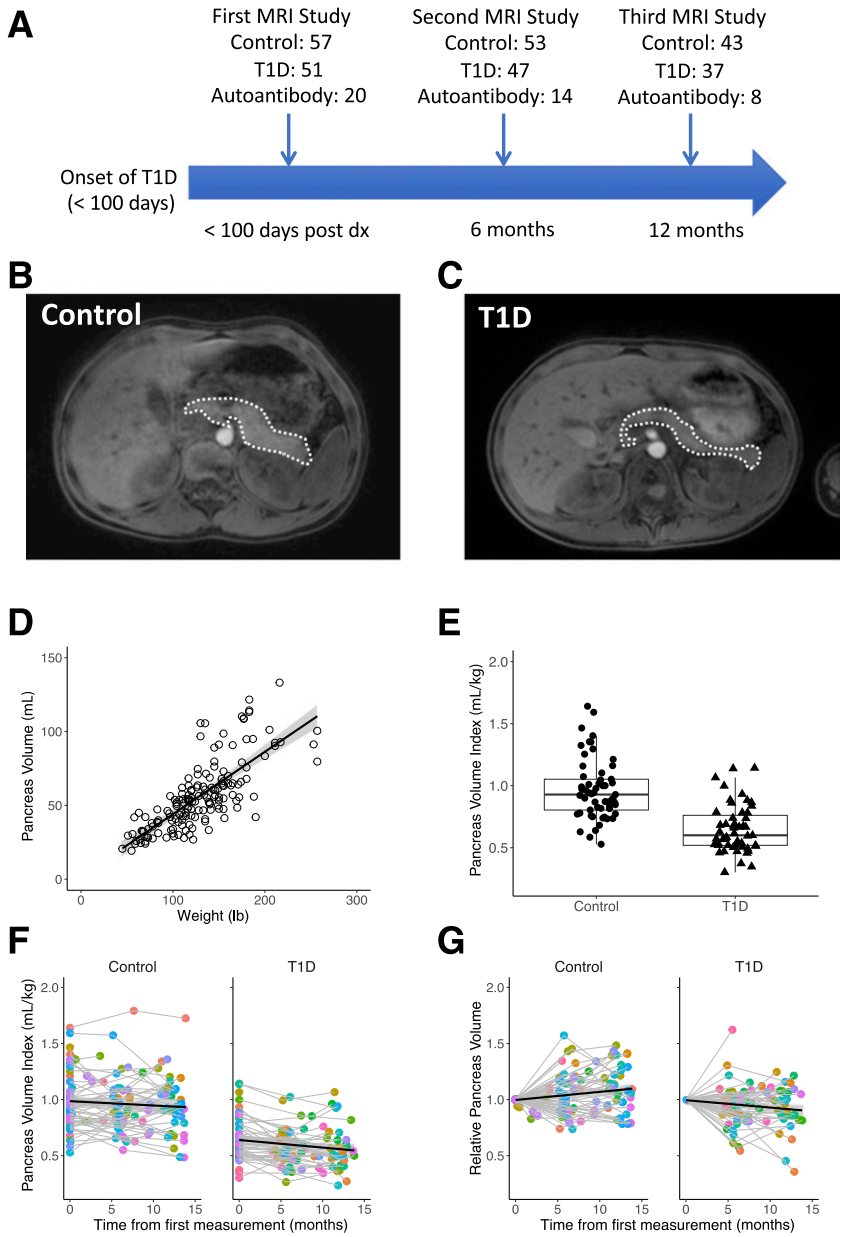

Figure 1—*A*: Schematic of study design. MRI was performed on participants with T1D within 100 days of diagnosis (dx) and repeated at 6-month increments. Participants with autoantibodies or without T1D were scanned at similar intervals. The number of individuals imaged at the 6- and 12-month time points is shown. *B*: An axial MRI slice through the abdomen of a control participant shows the pancreas (outlined in white). Participant was a 10-year-old male who weighed 29 kg. *C*: An axial MRI slice through the abdomen of a participant with recent-onset T1D shows a smaller, thinner pancreas than seen in the control participant (outlined in white). Participant was also a 10-year-old male who weighed 29 kg. *D*: In the control cohort, pancreas volume correlated with participant weight over the course of adolescent growth. *E*: Pancreas volume normalized by participant weight, yielding a pancreas volume index in units of mL/kg, is smaller in participants with T1D than control participants. *F*: Pancreas volume index is unchanged in the control pancreas over a 1-year period but declines in participants with T1D over the year after diagnosis. *G*: When normalized to the baseline measurement for each study participant, pancreas volume increases in the control population but declines in participants with T1D. Shading indicates the 95% CI in all figures.

MRI metrics interrogating pancreas microstructure (ADC), macromolecular content (MTR), inflammation (T2), and exocrine function (T1-weighted pancreas-to-spleen ratio) were performed at study baseline. A table summarizing the MRI techniques used, the quantitative MRI derived from each imaging technique, and the presumed pancreas pathology assessed by each MRI technique is shown in Fig. 3*A*. Pancreas ADC was different among the control, T1D, and autoantibody-positive participants (Fig. 3*B*) ($P < 0.05$). There was no apparent difference among the cohorts in average pancreas T2 (Fig. 3*C*) ($P = 0.75$), MTR (Supplementary Fig. 4*A*) ($P = 0.64$), or T1-weighted pancreas-to-spleen ratio (Supplementary Fig. 4*B*) ($P = 0.22$).

Visual inspection of ADC maps of the pancreas suggested that focal areas of the pancreas differed between the control and T1D pancreas (Fig. 3*D*). In order to examine differences between voxel-level ADC maps for the control and T1D participants at the initial time point, histogram analysis was performed for all pancreas voxels of control participants and participants with T1D. This histogram analysis (shown in Fig. 3*E*) revealed a difference in the distribution of voxels between the pancreas from control participants and participants with T1D at the initial time point. Participants with T1D had more voxels with higher ADC values in the pancreas ($P < 0.0001$, Kolmogorov-Smirnov test). Permutation tests of the ADC values of every voxel found a significant difference between the T1D and control distributions in the 25% quartile ($P = 0.01$), median ($P = 0.007$), and 75% quartile ($P = 0.02$). Collectively, these data indicate the presence of focal changes in the microstructure of the pancreas in T1D that may be assessed using DW-MRI.

CONCLUSIONS

The pancreas is known to be smaller in long-standing T1D; however, the dynamics of a decline in pancreas volume accompanying T1D are unknown, as there have been no longitudinal studies of pancreas volume in individuals with T1D or autoantibody-positive individuals. This study, for the first time to our knowledge, demonstrates a progressive decline in pancreas volume in individuals with T1D over the first year after diagnosis. Furthermore, DW-MRI detected focal areas of the diabetic pancreas with altered microstructure, which could correlate with areas of active disease. Our study included siblings of participants with T1D as control participants. Despite the fact that these sibling dyads share

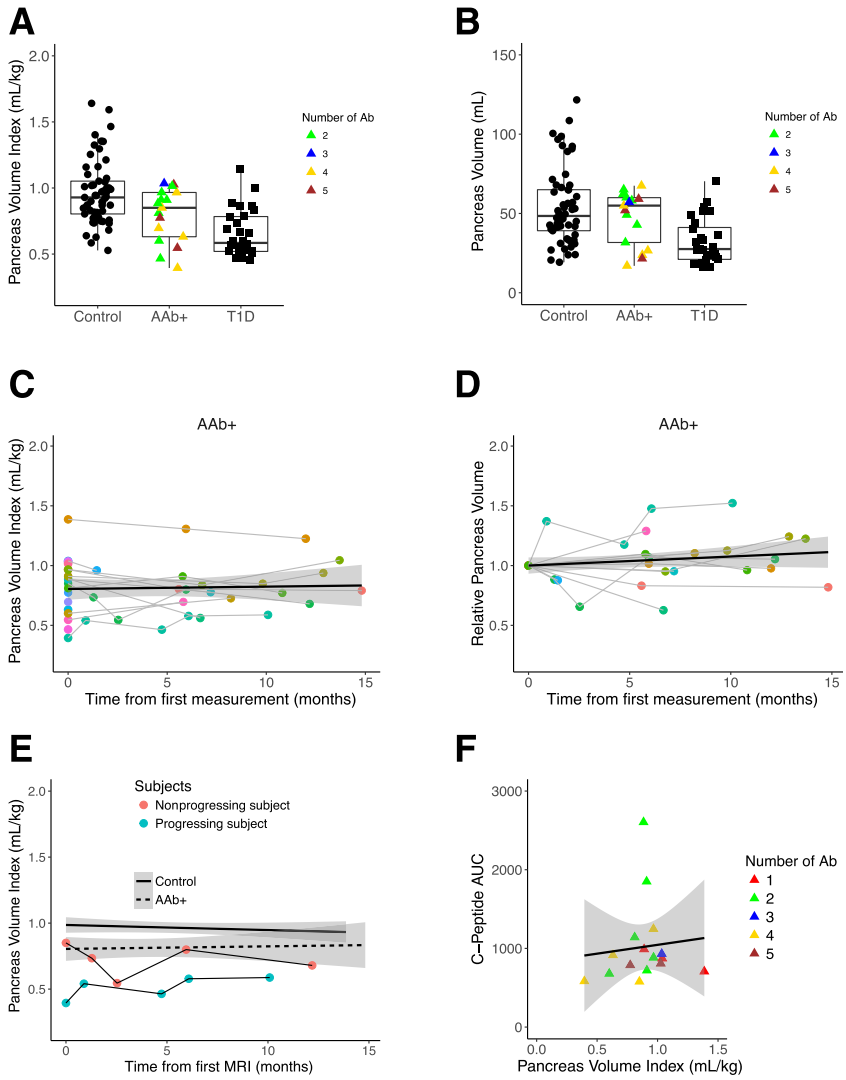

Figure 2—*A*: Pancreas volume index in autoantibody-positive (AAb+) participants with T1D is smaller than that in control participants and larger than that in participants with recent-onset T1D. Autoantibody-positive participants are color coded according to the number of autoantibodies present at the time of the MRI scan. *B*: Pancreas volume in autoantibody-positive participants demonstrates dichotomy with one group of participants with pancreas volume similar to that of control participants and one group with pancreas volume similar to that of participants with T1D. *C*: Average pancreas volume index displays no clear trend in the autoantibody-positive pancreas over a 1-year period. *D*: When normalized to the baseline measurement for each autoantibody-positive participant, pancreas volume displays no clear average trend over the course of the study. *E*: Two of the autoantibody-positive participants had abnormal glucose tolerance testing during the study period. One of these participants (denoted as the nonprogressing participant) remitted from stage 2 T1D to stage 1 T1D, whereas the other (denoted as the progressing participant) was diagnosed with stage 3 T1D. The solid black line indicates the average of the control cohort in this study, whereas the dashed black line indicates the average of the autoantibody-positive cohort in this study. *F*: Pancreas volume index does not correlate with insulin production in autoantibody-positive participants as assessed by OGTT. Shading indicates the 95% CI in all figures. Ab, antibody; AUC, area under the curve.

genetic background, the sibling with T1D had a lower average pancreas volume. The finding of ongoing pancreas atrophy in the first year after diagnosis with T1D suggests previously unknown ongoing pathophysiology that can be monitored by MRI in future trials.

The volume of the pancreas in control participants showed a considerable range among individuals. However, longitudinal MRI measurements demonstrated that pancreas volume, when normalized for participant weight, was stable in control participants over the course of normal adolescent growth. In contrast, these same measures declined in participants with T1D over the first year after diagnosis, demonstrating that pancreas growth is not keeping up with body weight increases in T1D. Relative changes in pancreas volume over the study year indicated that the pancreas grew, on average, 8.4% in control participants but shrunk by 7.2% in participants with recent-onset T1D, suggesting ongoing pancreas atrophy in recent-onset disease. In support of this finding, a longitudinal study of patients following pancreaticoduodenectomy found greater rates of decline in pancreas volume in patients who developed diabetes (28), suggesting that declines in pancreas volume may correspond with progression to T1D. The finding of large differences in pancreas volume between individuals demonstrates the difficulty in interpreting single measurements of pancreas volume and suggests that the trend in pancreas volume is most informative for monitoring disease progression. Pancreas volume index was not associated with A1C, presence of diabetic ketoacidosis at disease onset, or C-peptide production. Thus, pancreas volume may represent a biomarker that reports on aspects of T1D distinct from glycemic control or β-cell function that may not be captured in current disease monitoring.

In this study, we used a battery of four quantitative MRI techniques that can inform on aspects of tissue composition and structure. Notably, two of the techniques performed in this study have previously demonstrated sensitivity to exocrine dysfunction in pancreatitis: DW-MRI (16) and T1-weighted pancreas-to-spleen ratio (18). Of the four quantitative MRI techniques performed, only DW-MRI demonstrated a significant difference in the pancreas in T1D. This finding suggests alterations in microstructure in the pancreas in recent-onset T1D. Furthermore, T1D is thought to be a lobular disease (19), as previously suggested in MRI studies of inflammation using magnetic nanoparticles (29). In order to examine the heterogeneity across all voxels of the pancreas, histogram analysis was performed for the ADC of all T1D and control participants. This histogram analysis as well as permutation analysis demonstrated differences in the ADC of the pancreas between the control and T1D pancreas, which were not evident when averaging all values of the pancreas. This demonstrates the need for examining voxel-level

A

MRI Technique	MRI Metric	Possible Pancreas Pathology in T1D
Anatomical MRI	Pancreas Volume	Reduced Pancreas Volume
Diffusion-weighted imaging (DWI)	Apparent Diffusion Coefficient (ADC)	Reduced Structural integrity and cell density
Magnetization transfer (MT) MRI	Magnetization Transfer Ratio (MTR)	Fibrosis
T2 Mapping	Transverse Relaxation Time	Inflammation and edema

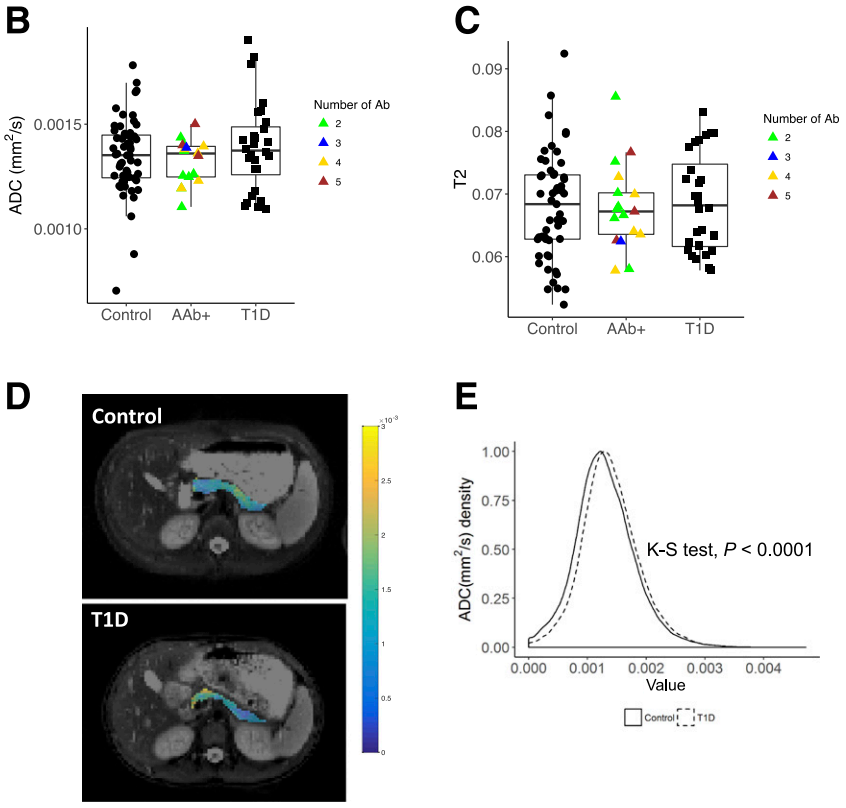

Figure 3—*A*: MRI techniques performed in this study, corresponding quantitative MRI metric, and possible pancreas pathologies in T1D that may be assayed by each technique. *B*: DW-MRI indicates that the mean ADC in the pancreas is higher in participants with recent-onset T1D. Autoantibody-positive (AAb+) participants are color coded according to the number of autoantibodies present at the time of the MRI scan. *C*: The mean T2 of the pancreas is similar in control participants, participants with T1D, and autoantibody-positive participants. *D*: Multiparametric maps of the ADC in the pancreas of a control participant (top) and participant with recent-onset T1D (bottom) reveal focal variations in ADC. The participants shown in *D* are both 10-year-old females; the control participant weighed 35 kg, and the participant with T1D weighed 36 kg. Note the difference in pancreas area between the participants and the focal area with high ADC in the head of the pancreas of the participant with T1D. *E*: Histogram analysis comparing the ADC of all voxels from control pancreata with the ADC of voxels from all T1D pancreata demonstrates differences in the distribution of ADC values, with higher numbers of voxels with high ADC in the T1D pancreas. Ab, antibody; K-S test, Kolmogorov-Smirnov test.

imaging data when characterizing imaging data from diseases with lobular presentation, such as T1D, rather than averaging all voxels. Furthermore, it demonstrates the need to analyze the entire pancreas, rather than performing region of interest analysis that may miss areas of focal changes. Neither MTR, T2, nor T1-weighted pancreas-to-spleen ratio displayed any changes in average values throughout the pancreas among control participants, participants with T1D, or autoantibody-positive participants, suggesting that these MRI techniques do not detect changes in the pancreas accompanying T1D or are not sensitive enough to detect changes. Notably, each of these techniques demonstrated large variations in repeat measurements in control participants, suggesting that further refinement of each technique is needed.

The period immediately prior to diagnosis with T1D may be a critical window to better characterize the natural history of the disease. Pancreas volume index in autoantibody-positive participants (stage 1 T1D) was found to be intermediate between that of the T1D and control cohorts. Pancreas volume index spanned a large range in the autoantibody-positive population, consisting of individuals with pancreas volume similar to that of control participants and others with pancreas volume similar to that of the T1D participants. Of this latter subset with a smaller pancreas, two participants progressed to stage 2 T1D over the course of this study. One of these autoantibody-positive participants experienced disease remission with a concurrent increase in pancreas volume, whereas the other progressed to stage 3 T1D and had the lowest pancreas volume index of the autoantibody-positive population in this study. The large range of pancreas volumes in the autoantibody-positive population in this study possibly reflects the heterogeneity in risk and progression of these participants toward T1D development (30). Studies are underway to perform MRI in additional autoantibody-positive participants as well as additional MRIs in currently enrolled autoantibody-positive participants.

Previous cross-sectional studies of pancreas size disagreed about whether there is an association between pancreas volume and T1D duration in adults (4,5,9–11). However, a single study in pediatric participants did find reduced pancreas volume with longer duration of disease (31). The different trajectories of pancreas volume in pediatric and adult-onset T1D may reflect proposed differences in T1D pathology (32). Notably, the increased severity of autoimmune destruction in childhood-onset T1D (33) may manifest in pancreas atrophy in children with recent-onset T1D. The large differences in pancreas volume between individuals with T1D highlights the difficulty in determining temporal associations using cross-sectional data and demonstrates the utility of longitudinal monitoring as performed in this study.

The MRI exam performed in this study was well tolerated by pediatric

participants as young as 8 years old. The MRI protocol was short, with the volumetric data collected in 25 s and the entire exam performed in 25 min. The ease of the exam was reflected in the low dropout rate of 7%. No participants halted the exam during scanning due to claustrophobia or other concerns. No contrast media was injected to avoid the risks of nephrogenic systemic fibrosis and brain retention recently attributed to MRI contrast agents (34). The speed, non-ionizing nature of MRI, and lack of need for contrast agent make this technique well suited for longitudinal scanning in pediatric populations.

This study is subject to several limitations. In this study, the pancreas was manually outlined by the same blinded radiologist, to avoid interreader variability, but measurements could be subject to intrareader variability. Pancreas segmentation algorithms are under development that may improve this potential source of error (35). Abdominal MRI suffers from respiratory motion that can impair accurate delineation of pancreas volume. Motion correction techniques are under development to improve abdominal MRI quality and improve the accuracy of pancreas volume measurements (36). Study participants were primarily children and adolescents and exhibited ranges of body weight and pubertal progression. We accounted for changes in body weight by using the pancreas volume index, but we did not assess pubertal status in participants. In an effort to enhance participant recruitment, autoantibody testing was not performed in every control participant. As the control participants included siblings of participants with T1D, there is a higher risk of incidence of T1D in these control participants. However, 16 of the 27 siblings in this study were autoantibody negative, and these participants had similar pancreas volume to the other 11 siblings with unknown autoantibody status. Furthermore, we would expect that inadvertently including control participants in the early stages of T1D would actually dilute the differences shown in this study. Our current sample sizes are limited, particularly in the autoantibody-positive cohort and their longitudinal progression as well as the two participants who progressed to stage 2 T1D over the course of the study. Further recruitment of this population is underway to image more individuals in this crucial window.

In conclusion, longitudinal volumetric MRI demonstrates a decline in pancreas volume in T1D over the first year of diagnosis. These findings raise fundamental questions that challenge our understanding of T1D pathogenesis and its β-cell–specific nature. The etiology of a decline in pancreas volume is unknown, but could stem from a loss of the trophic influence of insulin (37) or the inflammatory process related to insulitis (38). Further longitudinal studies are underway in the T1D and autoantibody-positive cohorts to fully characterize the time course of pancreas volume in the natural history of T1D. Of the quantitative MRI techniques used in this study, diffusion-weighted imaging may provide complementary information regarding the pathophysiology of the pancreas in T1D. Whether the MRI techniques described in this study will prove useful for monitoring response to immunomodulatory therapies warrants investigation.

Funding. These studies were supported by JDRF (3-SRA-2015-102-M-B), the Vanderbilt Institute for Clinical and Translational Research (UL1-TR-000445, KL2-TR-000447, and TR-002243 from the National Center for Advancing Translational Sciences/NIH), the Type 1 Diabetes TrialNet Clinical Center grant (U01-DK-085465 to W.E.R.), and a JDRF Career Development Award (to D.J.M.). This research was performed using resources and/or funding provided by National Institute of Diabetes and Digestive and Kidney Diseases grant DK-94199, the Vanderbilt Diabetes Research and Training Center (DK-20593), and the Department of Veterans Affairs. Some of the data used in this manuscript are in part a result of an ancillary study to the Type 1 Diabetes TrialNet Pathway to Prevention Study, which was funded by NIH grant U01-DK-085465.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. J.V., A.C.P., and D.J.M. designed the experiments, performed the research, recruited participants, analyzed the data, and wrote the manuscript. J.W., C.B, and J.J.W. performed the research and recruited participants. M.H. read and outlined the pancreas on all MRI images. L.D. and H.K. performed statistical analysis and data interpretation. W.E.R. and D.J.M. recruited participants and interfaced with TrialNet. All authors critically revised the article and approved the final version. J.V. 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 the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented at the JDRF Network for Pancreatic Organ Donors with Diabetes Annual Meeting, St. Pete Beach, FL, 22–25 February 2015; the 76th Scientific Sessions of the American Diabetes Association, New Orleans, LA, 10–14 June 2016; the 77th Scientific Sessions of the American Diabetes Association, San Diego, CA, 9–13 June 2017; and the 78th Scientific Sessions of the American Diabetes Association, Orlando, FL, 22–26 June 2018.

References

1. Chiarelli F, Verrotti A, Altobelli E, Blasetti A, Morgese G. Size of the pancreas in type I diabetic children and adolescents. Diabetes Care 1995; 18:1505–1506
2. Fonseca V, Berger LA, Beckett AG, Dandona P. Size of pancreas in diabetes mellitus: a study based on ultrasound. Br Med J (Clin Res Ed) 1985; 291:1240–1241
3. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965;14: 619–633
4. Gaglia JL, Guimaraes AR, Harisinghani M, et al. Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. J Clin Invest 2011;121:442–445
5. Williams AJ, Thrower SL, Sequeiros IM, et al. Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. J Clin Endocrinol Metab 2012;97:E2109–E2113
6. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964–1974
7. Campbell-Thompson M, Wasserfall C, Montgomery EL, Atkinson MA, Kaddis JS. Pancreas organ weight in individuals with disease-associated autoantibodies at risk for type 1 diabetes. JAMA 2012;308:2337–2339
8. Campbell-Thompson ML, Kaddis JS, Wasserfall C, et al. The influence of type 1 diabetes on pancreatic weight. Diabetologia 2016;59:217–221
9. Garcia TS, Rech TH, Leitão CB. Pancreatic size and fat content in diabetes: a systematic review and meta-analysis of imaging studies. PLoS One 2017;12:e0180911
10. Goda K, Sasaki E, Nagata K, Fukai M, Ohsawa N, Hahafusa T. Pancreatic volume in type 1 and type 2 diabetes mellitus. Acta Diabetol 2001;38: 145–149
11. Virostko J, Hilmes M, Eitel K, Moore DJ, Powers AC. Use of the electronic medical record to assess pancreas size in type 1 diabetes. PLoS One 2016;11:e0158825
12. Regnell SE, Peterson P, Trinh L, et al. Pancreas volume and fat fraction in children with type 1 diabetes. Diabet Med 2016;33:1374–1379
13. Saisho Y, Butler AE, Meier JJ, et al. Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. Clin Anat 2007;20: 933–942
14. Geraghty EM, Boone JM, McGahan JP, Jain K. Normal organ volume assessment from abdominal CT. Abdom Imaging 2004;29:482–490
15. Lee SS, Byun JH, Park BJ, et al. Quantitative analysis of diffusion-weighted magnetic resonance imaging of the pancreas: usefulness in characterizing solid pancreatic masses. J Magn Reson Imaging 2008;28:928–936

16. Balci NC, Momtahen AJ, Akduman EI, Alkaade S, Bilgin M, Burton FR. Diffusion-weighted MRI of the pancreas: correlation with secretin endoscopic pancreatic function test (ePFT). Acad Radiol 2008;15:1264–1268
17. Pazahr S, Blume I, Frei P, et al. Magnetization transfer for the assessment of bowel fibrosis in patients with Crohn's disease: initial experience. MAGMA 2013;26:291–301
18. Tirkes T, Fogel EL, Sherman S, et al. Detection of exocrine dysfunction by MRI in patients with early chronic pancreatitis. Abdom Radiol (NY) 2017;42:544–551
19. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2014;383:69—82
20. Mahon JL, Sosenko JM, Rafkin-Mervis L, et al.; TrialNet Natural History Committee; Type 1 Diabetes TrialNet Study Group. The TrialNet Natural History Study of the Development of Type 1 Diabetes: objectives, design, and initial results. Pediatr Diabetes 2009;10:97–104
21. Szczepaniak EW, Malliaras K, Nelson MD, Szczepaniak LS. Measurement of pancreatic volume by abdominal MRI: a validation study. PLoS One 2013;8:e55991
22. Chan E, Arlinghaus LR, Cardin DB, et al. Phase I trial of vorinostat added to chemoradiation with capecitabine in pancreatic cancer. Radiother Oncol 2016;119:312–318
23. Harms SE, Siemers PT, Hildenbrand P, Plum G. Multiple spin echo magnetic resonance imaging of the brain. Radiographics 1986;6:117–134
24. Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. NMR Biomed 2001;14:57–64
25. Greenbaum CJ, Harrison LC; Immunology of Diabetes Society. Guidelines for intervention trials in subjects with newly diagnosed type 1 diabetes [published correction appears in Diabetes 2003;52:2643]. Diabetes 2003;52:1059–1065
26. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–381
27. Cicchetti D. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychol Assess 1994;6:284–290
28. Yun SP, Seo HI, Kim S, Kim DU, Baek DH. Does the pancreatic volume reduction rate using serial computed tomographic volumetry predict new onset diabetes after pancreaticoduodenectomy? Medicine (Baltimore) 2017;96:e6491
29. Gaglia JL, Harisinghani M, Aganj I, et al. Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. Proc Natl Acad Sci U S A 2015;112:2139–2144
30. Knip M, Korhonen S, Kulmala P, et al. Prediction of type 1 diabetes in the general population. Diabetes Care 2010;33:1206–1212
31. Altobelli E, Blasetti A, Verrotti A, Di Giandomenico V, Bonomo L, Chiarelli F. Size of pancreas in children and adolescents with type I (insulin-dependent) diabetes. J Clin Ultrasound 1998;26:391–395
32. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M. A comparison of childhood and adult type I diabetes mellitus. N Engl J Med 1989;320:881–886
33. Poudel A, Savari O, Striegel DA, et al. Beta-cell destruction and preservation in childhood and adult onset type 1 diabetes. Endocrine 2015;49:693–702
34. Gulani V, Calamante F, Shellock FG, Kanal E, Reeder SB; International Society for Magnetic Resonance in Medicine. Gadolinium deposition in the brain: summary of evidence and recommendations. Lancet Neurol 2017;16:564–570
35. Bobo MF, Bao S, Huo Y, et al. Fully convolutional neural networks improve abdominal organ segmentation. Proc SPIE Int Soc Opt Eng 2018;10574:105742V
36. Sandrasegaran K, Lin C, Akisik FM, Tann M. State-of-the-art pancreatic MRI. AJR Am J Roentgenol 2010;195:42–53
37. Henderson JR, Daniel PM, Fraser PA. The pancreas as a single organ: the influence of the endocrine upon the exocrine part of the gland. Gut 1981;22:158–167
38. Nakanishi K, Kobayashi T, Miyashita H, et al. Relationships among residual beta cells, exocrine pancreas, and islet cell antibodies in insulin-dependent diabetes mellitus. Metabolism 1993;42:196–203