# Relative Pancreas Volume Is Reduced in First-Degree Relatives of Patients With Type 1 Diabetes

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## OBJECTIVE

Pancreas size is reduced in patients at type 1 diabetes onset and in autoantibody (AAB)-positive donors without diabetes. We sought to determine whether pancreas volume (PV) imaging could improve understanding of the loss of pancreas size in first-degree relatives (FDRs) of patients with type 1 diabetes. We also examined relationships among PV, AAB status, and endocrine and exocrine functions.

## RESEARCH DESIGN AND METHODS

We conducted a cross-sectional study that included five groups: $AAB^-$ control subjects (no diabetes and no first- or second-degree relatives with type 1 diabetes) ($N = 49$), $AAB^-$ FDRs ($N = 61$), $AAB^+$ FDRs ($N = 67$ total: $n = 31$ with a single positive AAB [$AAB^+$ single] and $n = 36$ with multiple positive AABs [$AAB^+$ multiple]), and patients with recent-onset type 1 diabetes (<1 year) ($N = 52$). Fasting subjects underwent 1.5T pancreatic MRI, and PV and relative PV (RPV) (PV-to-BMI ratio) were analyzed between groups and for correlations with $HbA_{1c}$, C-peptide, glucose, and trypsinogen.

## RESULTS

All FDR groups had significantly lower RPV adjusted for BMI ($RPV_{BMI}$) than control subjects (all $P < 0.05$). Patients with type 1 diabetes had lower $RPV_{BMI}$ than $AAB^-$ FDR ($P < 0.0001$) and $AAB^+$ multiple ($P \leq 0.013$) subjects. Transformed data indicated that trypsinogen levels were lowest in patients with type 1 diabetes.

## CONCLUSIONS

This study demonstrates, for the first time, all FDRs having significantly smaller $RPV_{BMI}$ compared with $AAB^-$ control subjects. Furthermore, $RPV_{BMI}$ was significantly lower in patients with recent-onset type 1 diabetes than in the $AAB^-$ FDR and $AAB^+$ multiple groups. As such, $RPV_{BMI}$ may be a novel noninvasive biomarker for predicting progression through stages of type 1 diabetes risk. This study highlights the potential paracrine relationships between the exocrine and endocrine pancreas in progression to type 1 diabetes in subjects at risk.

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Despite advances in medical diagnosis and treatment, type 1 diabetes remains one of the costliest diseases in the U.S. in terms of health care dollars spent for diabetes-related costs (1). Immunotherapy-based intervention trials carried out in patients with new-onset type 1 diabetes have demonstrated potential for short-term benefit (e.g., anti-CD20, anti-CD3, and cytotoxic T-lymphocyte–associated antigen 4 Ig) but have failed to demonstrate long-term efficacy (2,3). The inability to provide long-term preservation of β-cell function or prevent type 1 diabetes may stem from unanswered

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fundamental questions about the pathophysiology of type 1 diabetes in the preclinical stages.

Interest in pancreas size in diabetes has a long history. More than 100 years ago, autopsy studies by Cecil reported reductions in pancreas weight in 25 of 90 patients with diabetes (4). Additional autopsy studies reported variable losses in pancreas weights in patients with diabetes (5–7). As islets comprise only 1–2% of the fractional pancreas volume (PV), the reduction in PV is due to the significant loss of exocrine volume. The loss of pancreas weight has also been corroborated by radiologic imaging studies, including ultrasonography, computed tomography, and MRI, where PV were reduced by 31–52% in patients with long-standing type 1 diabetes and 26–31% in patients with recent-onset type 1 diabetes compared with control subjects (8–13). The reasons for this reduction are unknown and likely occurred months and years before clinical onset. Our previous study suggested that pancreas weight was reduced by 25% in organ donors with a single positive type 1 diabetes–related autoantibody (AAB) (14). This study suggested that reduced pancreatic size may parallel progressive loss of functional β-cell mass. Additional studies are underway to determine whether there is ongoing loss in pancreas size after onset of type 1 diabetes and whether volume loss is ameliorated by exogenous insulin. Studies have also shown a significant but non–clinically meaningful decline in exocrine function (low stool chymotrypsin or elastase) in patients with new-onset type 1 diabetes (15).

Potential explanations for the reduction in pancreas size in type 1 diabetes include loss of insulinotrophic effects on acinar cells and immunologic destruction of exocrine pancreatic tissue (16). Interestingly, pancreatic size is also reduced in fulminant type 1 diabetes, type 2 diabetes, and monogenic diabetes, implicating potentially overlapping mechanisms related to β-cell dysfunction (17–19). Other factors that may impact pancreas size are likely complex interactions between genetics, epigenetics, and environmental agents (20).

Advances in MRI technology continue to improve the quality of pancreas MRI interpretation, and MRI is an indispensable diagnostic tool for pancreatic disease (17,21,22). The purpose of this study was to characterize PV by MRI in first-degree relatives (FDRs) with and without islet-related AABs in comparison with AAB$^-$ control subjects with no family history of type 1 diabetes and patients with recent-onset type 1 diabetes. Correlations between PV and biomarkers for endocrine and exocrine functions were also tested.

## RESEARCH DESIGN AND METHODS

### Subject Enrollment

Control subjects and patients within a year of type 1 diabetes onset (recent onset) were enrolled through the University of Florida Diabetes Institute outpatient clinics. Type 1 diabetes diagnosis was assigned according to American Diabetes Association criteria to patients who were within 1 year of onset (23). The FDR subjects were recruited within the National Institutes of Health (NIH) TrialNet network and were categorized by AAB status (negative, a single AAB, or multiple AABs) (24). Subjects with a history of exocrine insufficiency or pancreatitis were excluded. All studies were performed in accordance with federal guidelines and regulations, and informed consent was provided based on University of Florida institutional review board approval (no. 201600705). Eligible subjects included females and males between the ages of 8 and 50 years who were willing to undergo imaging procedures and phlebotomy. Subjects in all groups were recruited throughout the duration of the study. Notably, our institutional review board approval allowed for inclusion of subjects with diabetes up to 50 years of age, while our analysis plan sought to limit the age range of control subjects and FDRs to those up to 45 years of age in accordance with the upper age limit for antibody screening in the TrialNet Pathway to Prevention Study. Subjects were interviewed for medical history and self-reported race, and body weight and height were measured and recorded. Pregnancy testing was performed in women of childbearing age. BMI was calculated as body weight in kilograms divided by the square of height in meters, and body surface area (Mosteller formula) was calculated as the square root of (height (cm) * body weight (kg)/3,600). Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Florida Clinical and Translational Science Institute (25).

### Radiology Protocols

Subjects were given detailed information regarding abdominal imaging without contrast or sedation. After a minimum 4-h fast to enhance differentiation of the pancreas borders, subjects underwent standard abdominal MRI. All MRI exams were performed on a 1.5T Siemens Avanto scanner (Siemens AG, Erlangen, Germany). The examination required a few breath holds and <45 min of exam time. The pancreas MRI protocol included triplanar localizer sequences, an axial nonfat saturated T1-weighted sequence for anatomic imaging and lesion characterization, axial T1-weighted fat saturated and nonfat saturated sequences to differentiate fat from pancreatic lesions, and an axial diffusion-weighted sequence (b500) for lesion detection (21). The parameters of the sequence, notably, the through-plane resolution and the 12-degree flip angle, were modified to increase contrast between the pancreas and surrounding tissues. A modified volumetric interpolated breath-hold examination (VIBE) sequence with a $320 \times 240$ mm$^2$ in-plane field of view and 3-mm slice thickness was created to provide a voxel resolution of $1.7 \times 1.0 \times 3.0$ mm$^3$. A total of 52 thin, contiguous slices was obtained centered over the region of the pancreas. The PV was calculated by manual delineation of the pancreas from each slice by one of three radiologists specializing in MRI imaging on off-line image-processing workstations (Vitrea FX, Toshiba Medical Systems, Tustin, CA, and Visage PACS, Visage Imaging, San Diego, CA) (Supplementary Fig. 1A and B). The MRI images were reconstructed into three-dimensional sequences providing detailed spatial resolution and greater fat and motion suppression, thereby improving contrast resolution and overall image quality. Interobserver agreement was measured by calculating the intraclass correlation coefficient (ICC) on data collected from three radiologists using five subjects' MRI scans. The ICC was calculated using a publicly available macro in SAS (26); an ICC value of 1 indicates perfect agreement. PV determinations in this small sample were found

to have high agreement (ICC 0.86 [95% CI 0.56, 0.97]).

## Laboratory Assays

Whole blood was collected the same day at the University of Florida Clinical and Translational Science Institute Clinical Research Center and shipped overnight on ice packs to Northwest Lipid Metabolism and Diabetes Research Laboratories (Dr. Santica Marcovina, Director; Seattle, WA) for $HbA_{1c}$ analysis. Plasma and serum samples were aliquoted and stored frozen at $-20°C$ until shipment to ARUP Laboratories (University of Utah, Salt Lake City, UT) for serum trypsinogen levels or to Northwest Lipids Research Laboratories for fasting glucose levels and C-peptide analyses.

## AAB Assays

Frozen serum aliquots were shipped to the University of Colorado Autoantibody Core (Dr. Liping Yu, Director; Denver, CO) for biochemical AAB analysis. AABs against islet-related autoantigens (i.e., GAD, insulinoma-associated antigen 2, zinc transporter 8, and insulin) were measured using radioimmunoassays as previously described (27).

## Statistical Analysis

Data are presented as mean ± SD or 95% CIs with $n$ = number of subjects. All tests were two-sided, and a significance level = 0.05 was used. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC). Relative PV (RPV) adjusted for BMI ($RPV_{BMI}$) was determined from PV (mL) divided by BMI. Plots of general variable trends were performed for associations between variables. A linear mixed model was fit to the data to compare mean $RPV_{BMI}$ across the groups, adjusting for observed age and sex differences among the groups while accounting for the nonindependence of data in related individuals, as members of the same family were recruited. Residual variance was allowed to differ across the groups in the model. The Kenward-Roger denominator degrees of freedom was used in making inferences on the differences between the groups. When an overall group comparison was observed, post hoc pairwise comparisons were made with a Tukey correction. A test for a linear trend in $RPV_{BMI}$ across the five groups was also performed. Assumptions of the model

(normality, linearity, homoscedasticity) were verified by examination of the studentized residuals. Sample size prevented a comprehensive adjustment for race/ethnicity, but observed differences in racial composition (in the control group) led to two sensitivity analyses: 1) individuals ≤30 years of age (still with adjustment for age and sex) and 2) non-Hispanic white subjects (adjustment for age and sex). Standard linear regression was used to assess the relationship between $RPV_{BMI}$ and duration of type 1 diabetes.

## RESULTS

### Subject Demographic Data Analyses and MRI Compliance

A total of 246 subjects were recruited. Twelve subjects did not meet inclusion criteria (no subgroup listed, $N$ = 1; no family history of type 1 diabetes, $N$ = 7; and patients with type 1 diabetes aged >45 years ($N$ = 4)). Five subjects did not undergo MRI, which included a family of three whose study visit coincided with scanner maintenance and two boys (10 and 11 years old) with recent-onset type 1 diabetes who declined to undergo the MRI procedure. Two hundred and twenty-nine subjects met inclusion criteria for this study between 2014 and 2017 as follows: control subjects (no diabetes and no first- or second-degree relatives with type 1 diabetes) ($n$ = 49), FDRs with no AABs ($AAB^-$ FDR) ($n$ = 61), FDRs with a single positive AAB ($AAB^+$ single) ($n$ = 31), FDRs with multiple positive AABs ($AAB^+$ multiple) ($n$ = 36), and patients with recent-onset type 1 diabetes ($n$ = 52) (Table 1). Overall, 44% of all study subjects were male, and mean ± SD age was 19.6 ± 10.5 years (Table 1). The range of diabetes durations was 0–363 days, with a mean of 175 ± 105 days. Radiologic procedures were well tolerated.

### Body Weight, BMI, PV, and $RPV_{BMI}$ by Age

Body weights (kg) and BMI increased with age in all groups, with greater variability of body weight in older subjects compared with those <20 years old (Supplementary Fig. 2A and B). The $AAB^+$ single group had the highest mean age (Table 1). PV showed similar increases with age except for patients with recent-onset type 1 diabetes, who had smaller PV (Supplementary Fig. 3). Both height and weight were significantly ($P$ < 0.05) and independently

associated with PV; we thus decided to use BMI in calculating $RPV_{BMI}$ (PV/BMI), as BMI is a standard measure with wide generalizability. $RPV_{BMI}$ showed group trends in comparison with age similar to those for PV in comparison with age. Patients with recent-onset type 1 diabetes had smaller $RPV_{BMI}$ within this age range (Fig. 1 and Table 1). Normalization of PV by body weight ($RPV_{BW}$ [Supplementary Fig. 4]), height, and body surface area mirrored data using the $RPV_{BMI}$ ratio by age (Table 1).

### Reduced $RPV_{BMI}$ in FDRs and Patients With Recent-Onset Type 1 Diabetes

There was a significant linear trend of $RPV_{BMI}$ across groups ($P$ < 0.0001) (Fig. 1 and Supplementary Table 1). Subsequent pairwise testing found that all FDR groups, independent of AAB status, had significantly lower $RPV_{BMI}$ than control subjects. Subjects with recent-onset type 1 diabetes had lower $RPV_{BMI}$ than control, $AAB^-$ FDR, and $AAB^+$ multiple subjects (all $P$ ≤ 0.01) (Fig. 2). There was, however, no statistical difference in $RPV_{BMI}$ between $ABB^+$ single subjects and subjects with new-onset type 1 diabetes ($P$ = 0.2651). These results were adjusted for age and sex, given group differences with respect to the distribution of these variables. In addition, we performed two sensitivity analyses: 1) individuals ≤30 years of age (still with adjustment for age and sex) and 2) non-Hispanic white subjects (adjustment for age and sex). While statistical significance was lost for some of the pairwise comparisons, due to a smaller sample size, overall trends of decreasing $RPV_{BMI}$ across groups remained (linear trend test remained statistically significant for both sensitivity analyses).

The distribution of PV was also examined with respect to absolute AAB number (i.e., 0, 1, 2, 3, and 4) (Supplementary Fig. 5 and Supplementary Table 2). Although sample sizes in each subgroup were small, in comparison of PV across each of the absolute AAB categories, subjects who had three or four AABs may have had smaller PV compared with that in FDRs with only one AAB as well as in those with two AABs. We also examined whether $RPV_{BMI}$ correlated with diabetes duration in the patients with recent-onset type 1 diabetes. $RPV_{BMI}$ was not linearly associated with duration ($R^2$ = 0.002) (Supplementary Fig. 6).

**Table 1—Clinical characteristics of the study group participants**

|  | Overall | Control subjects | AAB⁻ FDR | AAB⁺ single | AAB⁺ multiple | Recent onset |
|---|---|---|---|---|---|---|
| N (%) | 229 | 49 (21.4) | 61 (26.6) | 31 (13.5) | 36 (15.7) | 52 (22.7) |
| Male sex | 101 (44.1) | 20 (40.8) | 23 (37.7) | 9 (29.0) | 22 (61.1) | 27 (51.9) |
| Age (years) | 19.6 ± 10.5 | 18.4 ± 5.9 | 21.1 ± 11.7 | 28.8 ± 13.3 | 15.9 ± 10.7 | 16.2 ± 6.1 |
| Body weight (kg) | 61.0 ± 23.7 | 61.8 ± 17.8 | 63.0 ± 22.1 | 75.3 ± 31.0 | 49.9 ± 22.6 | 61.3 ± 23.0 |
| BMI | 23.2 ± 6.4 | 22.7 ± 4.8 | 23.6 ± 6.1 | 27.6 ± 8.6 | 20.0 ± 5.7 | 22.6 ± 5.9 |
| PV | 74.9 ± 31.8 | 88.2 ± 30.8 | 81.2 ± 29.8 | 89.3 ± 35.4 | 61.2 ± 28.0 | 55.7 ± 21.6 |
| RPV$_{BW}$ | 1.2 ± 0.4 | 1.4 ± 0.4 | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.2 ± 0.2 | 0.9 ± 0.2 |
| RPV$_{BMI}$ | 3.2 ± 1.1 | 3.9 ± 1.1 | 3.5 ± 0.9 | 3.3 ± 1.1 | 3.0 ± 1.0 | 2.5 ± 0.2 |
| RPV-to-height ratio | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.4 ± 0.1 | 0.3 ± 0.1 |
| Race/ethnicity |  |  |  |  |  |  |
| NH white | 177 (77.3) | 28 (57.1) | 52 (85.3) | 25 (80.7) | 29 (80.6) | 43 (82.7) |
| NH black | 11 (4.8) | 5 (10.2) |  | 1 (3.2) | 3 (8.3) | 2 (3.9) |
| NH Asian | 2 (0.9) | 1 (2.0) |  |  |  | 1 (1.9) |
| NH Am. Indian, Alaska Native | — | — | — | — | — | — |
| NH Hawaiian, Pacific Islander | 1 (0.4) | 1 (2.0) |  |  |  |  |
| Hispanic | 27 (11.8) | 6 (12.2) | 8 (13.1) | 4 (12.9) | 4 (11.1) | 5 (9.6) |
| NH multiple race/unknown | 11 (4.8) | 8 (16.3) | 1 (1.6) | 1 (3.2) |  | 1 (1.9) |
| Laboratory tests* |  |  |  |  |  |  |
| HbA$_{1c}$ (%) | 5.5 ± 1.0 | 5.0 ± 0.3 | 5.1 ± 0.3 | 5.2 ± 0.3 | 5.1 ± 0.4 | 7.0 ± 1.3 |
| HbA$_{1c}$ (mmol/mol) | 36.8 ± 11.2 | 31.5 ± 3.6 | 32.0 ± 3.3 | 33.5 ± 3.5 | 32.6 ± 3.9 | 52.6 ± 13.9 |
| Glucose (mg/dL) | 104.0 ± 37.5 | 90.0 ± 6.0 | 93.0 ± 6.7 | 96.0 ± 8.7 | 94.7 ± 16.0 | 141.4 ± 64.3 |
| C-peptide (ng/mL) | 2.0 ± 1.2 | 2.1 ± 0.7 | 2.3 ± 1.3 | 2.5 ± 1.2 | 1.9 ± 1.2 | 1.2 ± 0.9 |
| Trypsinogen (ng/mL) | 23.9 ± 18.9 | 23.4 ± 10.0 | 25.8 ± 12.4 | 38.2 ± 40.5 | 20.0 ± 8.6 | 16.3 ± 9.9 |

Data are mean ± SD or N (%). Am. Indian, American Indian; NH, non-Hispanic. *HbA$_{1c}$ (% and mmol/mol), n missing = 5; glucose, n missing = 0; C-peptide, n missing = 1; trypsinogen, exclusion of outlier n = 1 and missing for analysis = 16.

## Endocrine and Exocrine Cell Function Tests

Laboratory assays were complete on the 229 subjects except for five missing samples for HbA$_{1c}$, one missing sample for C-peptide, and one excluded data point for trypsinogen (Table 1). Patients with recent-onset type 1 diabetes had higher HbA$_{1c}$ and glucose levels and lower C-peptide levels compared with all other groups (Table 1). While the study was not powered to formally test across the study subgroups, we noted decreased trypsinogen levels (though all within reference ranges) in patients with recent-onset type 1 diabetes compared with other groups ($P \leq 0.029$) (Supplementary Table 4).
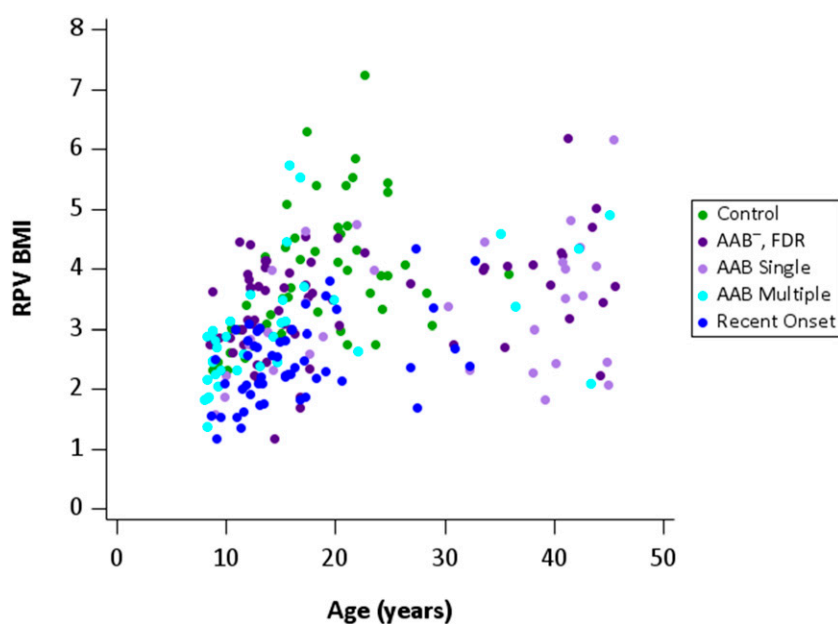
## CONCLUSIONS

This study showed for the first time, using noninvasive MRI, that AAB⁻ FDR subjects have significantly smaller RPV$_{BMI}$ than AAB⁻ control subjects. The data for AAB⁺ single subjects also corroborate our previous study that showed reduced pancreas weights in AAB⁺ single organ donors without diabetes (14). These findings bring renewed interest to the complexity of interactions between the pancreatic endocrine-exocrine compartments in type 1 diabetes and monogenic diabetes (28). They also raise a novel hypothesis that a smaller pancreas in FDRs may act as a predisposing factor for type 1 diabetes risk, particularly if exocrine and endocrine mass reductions occur in parallel. Mechanisms underlying a smaller pancreas are expected to result from complex genetic and environmental interactions that would affect pancreas growth rates and risk of progression to type 1 diabetes. Such interactions could include the in
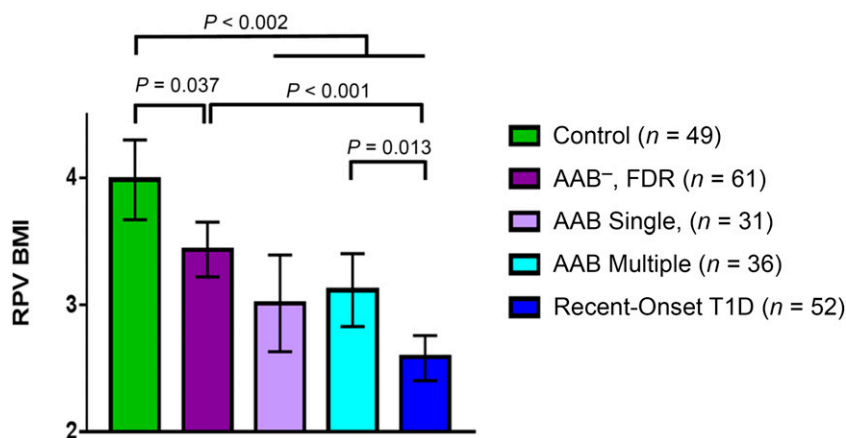


**Figure 1**—RPV$_{BMI}$ by age. RPV$_{BMI}$ increased with age to ~25 years of age in all study groups and then plateaued. Subjects in the group with recent-onset type 1 diabetes had the lowest RPV$_{BMI}$ trends by age.

**Figure 2**—Model-estimated mean (95% CI) $RPV_{BMI}$ by study group. Model-estimated mean $RPV_{BMI}$ was significantly different between control subjects and all other groups; a linear trend test was significant. The model adjusted for age and sex and accounted for individuals from the same family. Mean $RPV_{BMI}$ for $AAB^-$ FDR subjects was significantly lower than for control subjects and significantly higher than for patients with recent-onset type 1 diabetes (T1D). Mean $RPV_{BMI}$ for $AAB^+$ multiple subjects was significantly different from that for patients with recent-onset type 1 diabetes.

utero environment, childhood, and puberty, during which the majority of rapid organ growth rates occur. Genes modifying type 1 diabetes risk are known from 33-wide sequencing studies, with the HLA class II loci being major determinants of type 1 diabetes development and another ~40 non-HLA genes with modifying effects (29). HLA class II loci could impact exocrine innate immunity for environmental factors including pathogens such as viruses or bacteria. Future studies examining the potential relationships between HLA and PV are planned. In addition, subclinical exocrine inflammation has been reported in donors with type 1 diabetes and suggests that chronic subclinical pancreatitis could contribute to loss of pancreatic volume (30). Similarly, the subclinical exocrine insufficiency noted in patients with type 1 diabetes may be indicative of a pathogenic loss of exocrine tissue secondary to infection (30–34).

Saisho (35) demonstrated that PV increases with age, with a plateau at ~20 years of age, and eventually decreases after 60 years of age. Studies have also demonstrated a positive effect of BMI on pancreas size (36). In our study, this positive relationship between age and body or BMI was detected in all groups, with a similar plateau at ~25 years. Thus, our study confirms numerous previous studies showing that patients with type 1 diabetes have normal body growth rates. However, while the PV also

increased with age in control subjects and FDRs, PV and $RPV_{BMI}$ were lowest in patients with recent-onset type 1 diabetes, as found in previous studies (37). In our study, marked reduction in $RPV_{BMI}$ was present at onset of type 1 diabetes. However, $RPV_{BMI}$ was not linearly associated with diabetes duration in our population of patients with type 1 diabetes with duration of 0–363 days. In support of this notion, other cross-sectional studies have reported that PV or pancreas weight did not change with type 1 diabetes duration (38). In children with type 1 diabetes, PV determined by MRI did not correlate with diabetes duration but did correlate positively with total daily dose of insulin (39). Another MRI study in adults with recent-onset type 1 diabetes showed no correlation of PV with diabetes duration, glucose or C-peptide levels, $HbA_{1c}$, or AAB status (12). These studies contrast with a meta-analysis that was conducted from three studies of patients with type 1 diabetes with a decrease in PV with longest durations, albeit with very high variability between studies (37). Another study was conducted in patients with type 1 diabetes ranging in age from 4 to 67 years, and analysis of MRI and computed tomography images showed a significant loss of $RPV_{BW}$ with age as well as a reduction in RPV with diabetes duration (40). Conflicting findings between studies could be due to methodological differences, including radiology

technique and whether the radiographic analysis was performed during fasting or fed conditions. Analysis during fed conditions could interfere with pancreas delineation from adjacent soft tissues, including stomach and small intestines. As with many clinical parameters, individual variability in PV is found, and, thus, longitudinal studies would be particularly informative in a given subject to determine changes relative to baseline. Such data would be informative in developing accurate risk assessments for subjects with stage 1 or stage 2 type 1 diabetes and establishing evaluable responses to interventional therapies in addition to standard end points, such as preservation of C-peptide. It may also be inferred from the results of Regnell et al. (39) that resolution of insulinopenia through insulin therapy stopped further decreases in PV in children with type 1 diabetes. Our observations of decreased $RPV_{BMI}$ in $AAB^-$ FDRs with further decreases in $AAB^+$ single and $AAB^+$ multiple subjects suggest to us additional mechanisms leading to loss of PV in addition to subclinical insulinopenia. This notion is further supported by the fact that $RPV_{BMI}$ did not correlate with several measures of β-cell function, including $HbA_{1c}$, C-peptide levels, and glucose levels, in FDR subjects.

Recent studies indicate that there may be autoimmune infiltration of the exocrine portion of the pancreas, and AABs to exocrine proteins have been reported in patients with type 1 diabetes (20,31,41). Exocrine dysfunction has been reported and indicated by reduction in trypsinogen levels in patients with type 1 diabetes and $AAB^+$ multiple subjects without diabetes (42). While our study was not powered to make formal comparisons across groups, we observed a trend of lower trypsinogen levels in $AAB^+$ multiple subjects and in patients with recent-onset type 1 diabetes, which highlights subclinical exocrine dysfunction in type 1 diabetes. Notably, there was no apparent reduction in trypsinogen among $AAB^-$ FDR and $AAB^+$ single versus control subjects despite the apparent reduction in $RPV_{BMI}$. As reductions in trypsinogen were within typical reference ranges, trypsinogen levels may not be as sensitive as or correlate with RPV measurements in the earliest stages of type 1 diabetes. Whether loss of trypsinogen

secretion occurs secondary to decreased insulinotropic effects on the exocrine portion or from a direct autoimmune insult to the acinar cells or a combination of both remains unknown. Further studies would be of interest to elaborate the exocrine autoimmune process in the preclinical phase of type 1 diabetes.

New biomarkers are needed to improve monitoring of progression toward type 1 diabetes. Preventive trials have not provided long-term beneficial outcomes. Heterogeneity during stage 1 and stage 2 type 1 diabetes likely contributes to variable patient responses and may be partly responsible for poor outcomes to date in current clinical trials. Surrogate biomarkers of $\beta$-cell function do exist but are relatively insensitive. Imaging studies that can directly assess $\beta$-cell mass are still not available for clinical use (43–46). Our study supports the use of abdominal MRI to quantify $RPV_{BMI}$ in subjects at high risk for type 1 diabetes to understand intersubject heterogeneity, changes over time, and association with numbers of AAB. This examination can be readily incorporated with other screening tests while monitoring progression to type 1 diabetes in FDRs. Furthermore, determination of $RPV_{BMI}$ may be valuable in patients after type 1 diabetes onset. For example, patients with smaller $RPV_{BMI}$ may have increased risk for higher glucose variability. Longitudinal studies would be most valuable in extending the current cross-sectional findings but would also be valuable to determine the normal range of $RPV_{BMI}$ variation in a given person over months and years. The rate of decline in $RPV_{BMI}$ may then lead to better prognosis for risk of progression from normoglycemia to dysglycemia and then clinical onset. Additional studies are needed in order to determine whether $RPV_{BMI}$ can be used as a predictive marker in progression to type 1 diabetes. Such studies are expected to confirm the utility of PV-based biomarkers in subjects at high risk and inform the stratification of subjects into future prevention trials.

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