Biological sex affects human islet gene expression and mitochondrial function in type 2 diabetes

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Abstract

The clinical characteristics of type 2 diabetes (T2D) differ between the sexes. For example, the risk of T2D is higher in males than in premenopausal females, whereas the risk of T2D-associated cardiovascular disease is higher in females. The sex-dependent mechanisms of T2D pathogenesis remain incompletely understood. Publicly available human islet datasets, such as HPAP and Humanislets.com, offer a valuable tool for uncovering the impact of biological sex on islet structure, gene expression, and function at a scale that was not previously possible. We performed an integrated analysis of data from publicly available sources to identify sex differences in baseline islet characteristics in donors without diabetes and subsequently examined these features in donors who lived with T2D. Among donors without diabetes, female islets had a greater proportion of alpha-cells compared with male islets and showed enriched expression of ribosomal and mitochondrial pathways in both beta- and alpha-cells. Measurements of mitochondrial function in female islets revealed lower spare respiratory capacity compared to male islets due to higher basal respiration. Male and female islets had distinct changes in gene and protein expression in the context of T2D with female islets having greater preservation of insulin content and fewer defects in islet function. Together, these data show female islets have fewer islet impairments in T2D. This highlights the need for detailed mechanistic studies in both sexes to support effective and sex-informed interventions for T2D.

Introduction

Biological sex influences type 2 diabetes (T2D) incidence, complications, and treatment efficacy. There is a higher diabetes risk in men until approximately 50-60 years of age [1–4]. This male bias has been observed across all sociodemographic indices [5], though we note a female bias in some populations [6–8]. Relative protection against diabetes in pre-menopausal women has been attributed in part to the protective effects of estrogen [9,10]. Despite the lower incidence of T2D in premenopausal women, women who live with diabetes are at a higher risk of most T2D complications (e.g. cardiovascular and renal) compared to men [11–18]. Men and women with T2D also show differential responses to commonly used medications: male sex predicts better HbA1c-lowering efficacy of sulfonylureas, which stimulate beta-cell insulin secretion [19,20], and SGLT2 inhibitors, which decrease renal glucose reabsorption [20,21], whereas insulin-sensitizing thiazolidinediones and GLP-1 receptor agonists (GLP-1RAs) have greater HbA1c-lowering effects in females [20–24].

Sex differences in clinical observations are likely due to a combination of differences in insulin sensitivity and pancreatic beta-cell function. Improved insulin sensitivity in females is well-established and conserved from humans [25] to mice [26] to fruit flies [27]. Recent studies suggest that sex differences in pancreatic islets may also play a role. In mice, female islets show greater resilience to endoplasmic reticulum stress than male islets and have higher expression of genes involved in protein synthesis [28,29]. Sex differences in electrical activity have also been observed in mouse islets: glucose stimulation in female beta-cells results in lower potassium currents leading to a more depolarized membrane potential, more frequent burst-type action potentials, and smaller glucose-induced transient calcium increases [30,31]. HFD feeding in mice also causes sex-dependent transcriptomic changes [28], and female mice are better able to adapt to this metabolic stress through alpha-to-beta cell communication to maintain beta-cell calcium dynamics [32]. In humans, as in mice, female islets show enriched expression of pathways related to protein synthesis [29,33]. Beta-cell gene expression is closely linked to

epigenetic modifications, which differ between sexes on both sex chromosomes and autosomes, including at T2D candidate genes [34,35]. *Ex vivo* insulin secretion studies have also suggested that female islets may secrete more insulin than male islets in response to high glucose [29,33,34]. T2D causes profound changes in gene expression among islets from both sexes, and these changes are also partly sex-dependent [33,35,36]. However, our understanding of the molecular and functional changes that occur with T2D in female and male islets remains far from complete.

The Human Pancreas Analysis Program (HPAP) consolidates data from donor human islets across multiple institutes. The data collected through this program are uploaded to the publicly available PancDB database, and include measurements of human islet calcium imaging, mass cytometry, islet perifusion, oxygen consumption, and scRNA-seq, among others [37,38]. Humanislets.com is a repository with functional and omics data on a largely distinct pool of donors, including a wider range of perifusion experiments and a large human islet proteomics dataset [39]. Importantly, the datasets have enough donors to test for sex differences in many available outcomes.

Our analysis of HPAP and Humanislets.com revealed pronounced sex differences in islet composition, RNA and protein levels, and mitochondrial function in islets from donors without diabetes. We further find that gene and protein expression are differentially altered in T2D depending on biological sex, and that male islets exhibited mitochondrial defects that were absent from female islets among donors with T2D. Together, these data demonstrate clear differences in diverse islet characteristics between male and female islets among individuals without T2D, and show sex-dependent changes to islet biology in individuals who lived with T2D.

Materials and Methods

Data sources

Publicly available data were downloaded from the Human Pancreas Analysis Program (HPAP) Database, consortia under Human Islet Research Network (RRID:SCR_014393, https://hpap.pmacs.upenn.edu/) [37,38] (NIH grant numbers UC4-DK112217 and UC4-DK112232, RRID:SCR_016202), and Humanislets.com [39], an initiative of the Alberta Diabetes Institute IsletCore funded by the Canadian Institutes of Health Research, Breakthrough T1D Canada, and Diabetes Canada (5-SRA-2021-1149-S-B/TG 179092). Specific datasets used are described in detail below. To study sex differences within the healthy islet state, we specifically focused on donors without diabetes and aged 15-39 years. This age bracket was chosen to determine sex differences prior to the onset of menopause [40] and was used to compare male and female control (non-diabetic) donors only. However, when comparing across control and T2D for males and females, donors of all ages were included. Donors with type 1 diabetes were not included in our analyses due to insufficient donor numbers to support sex-specific analysis. Histograms displaying key metadata variables in each dataset are shown in Figure S1.

Cell type composition

Islet endocrine cell type composition data were available from both HPAP and Humanislets.com. CyToF data [41] estimating cell counts for each cell type were obtained from donor metadata available on HPAP, from which endocrine cell type proportions were calculated as described in the CyTOF Staining Workflow and Protocol on HPAP (https://hpap.pmacs.upenn.edu/explore/workflow/imaging-mass-cytometry?protocol=1, protocol accessed 23 September 2025, last updated 11 January 2021). Donors with a total endocrine cell count less than 30-fold lower than the geometric mean of all donors were excluded, as in [42]. On Humanislets.com, endocrine cell type proportions were estimated based on deconvolution of whole islet proteomics data [39] and were downloaded directly.

Gene expression analysis

Gene expression data from HPAP and Humanislets.com were analyzed to compare the sexes. Transcriptomic data from HPAP included scRNAseq data. To analyze scRNAseq data, beta-cell and alpha-cell expression were first pseudobulked to generate one gene expression profile per donor per cell type using the *Seurat* package [43]. Pseudobulking overcomes the confounding effects of uneven cell numbers per donor and appropriately treats individual donors, rather than cells, as independent observations. For Humanislets.com, only bulk islet RNAseq was analyzed by sex due to low n's available for scRNAseq data. Bulk islet proteomics data [44] were also downloaded and analyzed from Humanislets.com. Three comparisons were performed: female vs male among donors without diabetes aged 15-39, female control (without diabetes) vs female T2D across all ages, and male control vs male T2D across all ages. For all differential expression analyses, age was included as a covariate. The *ComplexUpset* R package was used for the UpSet plot.

DESeq2 [45] was used to analyze HPAP pseudobulk beta-cell and pseudobulk alpha-cell with apeglm shrinkage of effect size [46]. For Humanislets.com RNAseq data, pre-processed data were used, which already had batch-correction, and comparisons were performed using ANCOVA with age as covariate and Benjamini-Hochberg correction for multiple testing. For Humanislets.com proteomics data, *limma* [47] was used to compare female and male control donors aged 15-39, correcting for age. Gene Set Enrichment Analysis (GSEA) was performed on differential expression analysis output to identify key pathways using the clusterProfiler package [48], in which the direction signed -log10 p-values were used as the rank scores of the genes. To improve pathway visualization, pathways were considered redundant if their core enrichment genes exceeded a Jaccard index of 0.6. When selecting top pathways, redundant pathways were first refined by omitting the pathway with fewer gene components.

Insulin content

Islet insulin content data were downloaded from perifusion datasets on HPAP. There were two sets of islet perifusion data with corresponding insulin content data. The data from the University of Pennsylvania (UPenn) were presented per islet using a lysis buffer containing EDTA and detergent [49,50]. The data from Vanderbilt University were presented per islet equivalent (IEQ), using acid-ethanol extraction to lyse cells and isolate insulin. Acid-ethanol extraction is widely used in the field to extract insulin from biological samples at maximum yields [51–55], leveraging the strong acid to avoid the insulin precipitation pH range of ~4.5-6.5 [52,56] and ethanol to minimize the contaminating influences of exocrine impurities [57,58] while retaining insulin in solution [59]. Humanislets.com also used acid-ethanol extraction and presented insulin content data per IEQ. These data were obtained from the provided metadata.

Oxygen consumption

Islet oxygen consumption in response to stimuli was available from both HPAP and Humanislets.com. On Humanislets.com, oxygen consumption data were obtained using a Seahorse Bioanalyzer (Agilent) and calculated parameters were directly downloaded (spare respiratory capacity = maximum uncoupled respiration – minimum non-mitochondrial respiration, response to high glucose = mean respiration in 16.7 mM glucose – mean respiration in 3 mM glucose, baseline = last basal respiration) [39]. As three readings per donor were available, outliers were identified using Grubb's test and the average per donor was calculated. On HPAP, these data were obtained using a chemical oxygen probe (Oxyphor G3). Raw data were downloaded and parameters were calculated using the same formulae as used on Humanislets.com.

Intracellular calcium

Intracellular calcium imaging data using the ratiometric dye Fura-2 were available only from HPAP. Raw data were downloaded and processed as follows: 1) only regions that responded to high glucose were included (response counted if 16.7 mM glucose stimulated an increase in baseline-corrected calcium signal of at least 50%); 2) after filtering for glucose response, signals from multiple regions for each run were averaged; 3) runs without a specified time of first stimulus addition or without high glucose were excluded; 4) to account for different time periods between stimuli, only the first x minutes after stimulus addition were counted for each run, where x is the minimum period of time between that stimulus and the next, for any of the runs; 5) responses were averaged for each individual donor and summary statistics were calculated.

Dynamic hormone secretion

HPAP provides dynamic hormone secretion data from islet perifusion experiments performed at two sites: UPenn and Vanderbilt. For the UPenn data, areas under the curve (AUC) were calculated for the periods of perifusion during which islets were exposed to each stimulus, for both insulin and glucagon secretion. For the Vanderbilt data, there was a visible delay between stimulus addition and response. Therefore, the AUC calculated for each stimulus was determined based on the time course tracings: 12-39 min for 16.7 mM glucose, 63-87 min for 16.7 mM glucose + IBMX, 93-111 min for 1.7 mM glucose + epinephrine, and 123-138 min for KCl. Humanislets.com contains islet perifusion data measuring insulin secretion in response to glucose, leucine, and a mix of the free fatty acids oleate and palmitate [44]. For each database, AUC were calculated for the periods of perifusion during which islets were exposed to each stimulus.

Statistics

Unless otherwise specified, to determine statistical differences due to sex and disease, ANCOVA testing was performed with age as covariate. Multiple comparisons were performed

using Tukey's correction and the *emmeans* R package. For comparing distributions in Figure S1, the two-sample Kolgomorov-Smirnov test was used. Correlation of two numerical variables was performed using Pearson's correlation. Unless otherwise mentioned, a *p*-value threshold of 0.05 (or adjusted *p*-valued threshold where relevant) was used. All relevant script for data processing and graphing are available at https://github.com/singyoungchen/sex-differences-human-islet-characteristics-.

Results

Female islets contain a greater proportion of alpha-cells and a smaller proportion of beta-cells

We first examined islet cell type composition in male and female donors without T2D aged 15-39 years. Female islets had a significantly higher alpha-cell proportion compared to male islets in both the HPAP and Humanislets.com datasets (Figure 1A-B). Beta-cell proportion, in contrast, was lower in female islets compared with male islets, a difference that was statistically significant in the Humanislets.com data and which showed a non-significant but similar trend (p=0.11) in HPAP (Figure 1A-B). When we considered donors of all ages, however, the trends we observed were not consistent between the HPAP CyToF results and the Humanislets.com cell type proportion estimates. The HPAP data suggested that, among donors without diabetes of all ages, female islets showed higher alpha-cell (p<0.05) and lower beta-cell (p=0.072) proportions, consistent with observations from donors in the 15-39 age bracket (Figure 1C). In contrast, when including data for donors of all ages from Humanislets.com, we observed no sex difference in endocrine cell proportions between female and male donors without T2D (Figure 1D).

We next considered endocrine cell proportions in donors who lived with T2D. The HPAP data showed that T2D was associated with a trend for an increase in alpha-cell proportion (p=0.08) and a significant decrease in beta-cell proportion (p<0.05) in males but not females (Figure 1C). In contrast, the Humanislets.com data showed an increase in alpha-cell proportion and a

decrease in beta-cell proportion in female, but not male, donors who lived with T2D (Figure 1D). While the reason for this discrepancy is unclear, it may reflect the different techniques used by each team to obtain cell proportion estimates (antibody-based identification in HPAP, estimation based on marker protein expression data in Humanislets.com). Thus, while alpha-cell proportion was elevated among females in young donors without diabetes, we observed no consistent sex difference in donors who lived with T2D.

Islets from young female donors show higher expression of protein synthesis genes

We next checked for sex differences in baseline gene expression among islets from donors aged 15-39 without diabetes. Three RNAseq datasets were analyzed: pseudobulk beta-cell scRNAseq from HPAP, pseudobulk alpha-cell scRNAseq from HPAP, and bulk islet RNAseq from Humanislets.com. After conducting differential expression analysis to compare female and male donors (Table 1, S1), we performed GSEA. We found many female-biased pathways but relatively few male-biased pathways (Figure 2A-B, Table S2). In particular, pathways related to ribosomal biogenesis and protein synthesis showed a strong female bias in beta-cells and whole islets (Figure 2A, Table S2). The potential for enhanced protein synthesis capacity in female islets has previously been shown by us [29], and others [33], and is likely associated with the effects of estrogen in alleviating ER stress and preserving protein synthesis [60–63]. Mitochondrial pathways were also female-enriched (Figure 2A, Table S2), with very few male-biased pathways identified (Figure 2B, Table S2). The HPAP scRNAseq data also allowed us to perform sex-based analysis of alpha-cell transcriptomes. As with beta-cells, alpha-cells showed a female-biased enrichment of ribosomal and mitochondrial gene pathways (Figure 2A). “Histone demethylase activity” was the only significantly enriched pathway in male alpha-cells compared with female alpha-cells (Figure 2B).

Humanislets.com includes, to our knowledge, the largest open-source whole islet proteomics dataset currently available. We conducted differential expression analysis to compare protein

levels in female and male donors without diabetes aged 15-19 (Table S3) followed by GSEA. Wfound female-biased abundance of proteins related to ribosomes and mitochondria, as well as lipid transport and the plasma membrane (Figure 2C, Table S4). Male-biased pathways included those related to chromatin modification and transcriptional regulation (Figure 2C, Table S4). Thus, the female bias in expression of proteins related to protein synthesis and mitochondrial function was highly consistent between our RNAseq and proteomics data.

T2D is associated with differential shifts in gene and protein expression depending on sex

We next compared islet gene expression between donors with and without diabetes to evaluate any sex bias in gene expression changes associated with T2D. In the HPAP beta-cell scRNAseq dataset, 13 genes were significantly altered with T2D in females and 34 in males (Table 2, S5-6). In the Humanislets.com bulk RNAseq dataset, 0 genes were significantly altered with T2D in females and 583 in males (Table 2, S5). GSEA identified more pathways significantly altered with T2D in males than in females in beta-cells and whole islets (Table 3, S7). Among the pathways increased with T2D in female islets were pathways related to transcriptional regulation and histone modification (Figure 3A, Table S7; Figure 2B-C). In male islets, pathways related to cytosolic ribosomes increased with T2D in beta-cells and whole islets (Figure 3B, Table S8). Pathways decreased with T2D in female islets were dominated by pathways such as ribosomal and mitochondrial gene pathways (Figure 3C, Table S7). In males, T2D was associated with a decrease in beta-cell pathways related to vesicle processing and secretion, the extracellular matrix, and cell differentiation (Figure 3D, Table S8). Our data therefore suggest that there were distinct T2D-associated changes in gene expression between the sexes in beta-cells and whole islets.

The ability to distinguish beta-cell and alpha-cell gene expression in the HPAP scRNAseq dataset provides additional cell type-specific insights. GSEA showed that T2D was associated with significant enrichment of 345 pathways in females and 75 pathways in males (Table 3, S7-

8). Among top pathways across the 3 transcriptomic datasets in female islets, T2D was associated with an increase in pathways associated with transcriptional regulation and a decrease in ribosomal and mitochondrial pathways in alpha-cells, similar to changes observed in beta-cells (Figure 3A,C). In males, a smaller proportion of top pathways altered in T2D were identified in the pseudobulk alpha-cell scRNAseq dataset, suggesting T2D was associated with greater changes in alpha-cell gene expression in female islets than in male islets.

At the protein level, T2D was associated with altered expression of 441 proteins in female islets and 482 proteins in male islets, among which 230 were common between sexes and changed in the same direction (Figure 4A, Table S9-10). While no proteins changed in opposite directions, many were only significantly altered with T2D in only one sex (Figure 4A). GSEA showed that T2D was associated with enrichment of pathways related to cell adhesion and cell death in both sexes (Figure 4B-C, Table S11-12). In female islets, T2D was also associated with enrichment of pathways related to the inflammatory response and cytoskeleton organization (Figure 4B, Table S11). In male islets, T2D was associated with an enrichment of cytosolic ribosomal pathways (Figure 4C, Table S12), consistent with RNAseq data (Figure 3C, Table S8). Pathways that were decreased with T2D were similar between males and females, and included those related to vesicle trafficking and exocytosis, as well as mitochondrial components (Figure 4B-C, Table S11-12). Taken together, our analysis reveals sex differences in both islet gene and protein expression in donors without diabetes and individuals who lived with T2D.

Female islets may show greater preservation of insulin content than male islets in T2D

Healthy beta-cells have a remarkable capacity to rapidly increase insulin biosynthesis upon glucose stimulation [64]. In the context of T2D, islet insulin content has often [65], but not always [44,66], been reported to decrease. Given the sex differences in ribosome- and protein synthesis-related pathways, we compared islet insulin content in male and female islets.

Among donors without diabetes aged 15-39, no significant sex difference was observed in the Vanderbilt and Humanislets.com datasets, which used the acid-ethanol method to obtain insulin content data, including from detergent resistant granules (Figure 5A-B). In the UPenn data, however, a detergent-based method was used to obtain insulin content, possibly reflecting more recently synthesized insulin and less granule-stored insulin. The UPenn data showed a trend (p=0.068) toward higher insulin content in female islets (Figure 5C). Among donors without diabetes of all ages, Vanderbilt and Humanislets.com data found no sex difference in insulin content (Figure 5D-E), whereas UPenn insulin content data showed significantly higher islet insulin content in females (Figure 5F). Thus, insulin solubilized using the detergent method showed higher insulin content in female islets among donors without diabetes, but not insulin solubilized by acid-ethanol.

With respect to the effect of T2D on islet insulin content, data from Vanderbilt and Humanislets.com data showed that T2D was associated with increased insulin content in females (Figure 5D-E). This finding was not reproduced in the UPenn data, in which T2D was associated with decreased insulin content among female donors (Figure 5F). Male donors who lived with T2D did not have significantly altered islet insulin content compared to male donors without diabetes across all three datasets (Figure 5D-F). Therefore, T2D is associated with increased acid-ethanol-solubilized insulin but decreased detergent-solubilized insulin specifically in female islets.

Islets from female donors who lived with T2D have preserved mitochondrial activity

We next examined whether sex differences were present in islet oxygen consumption rate (OCR) data, which reflects mitochondrial function [67–69]. Consistent with higher mitochondrial gene expression in female islets, both HPAP and Humanislets.com showed that female islets from donors aged 15-39 without diabetes had a non-significant trend toward higher basal respiration than male islets (Figure 6A-B, E-F). Maximal respiratory capacity was not different between the

sexes (Figure 6C, G). Spare respiratory capacity refers to the difference between maximal respiratory capacity and basal respiration and reflects cells' ability to ramp up respiration in response to increased energy demand. Measurements of spare respiratory capacity showed that female islets had non-significant trends for lower spare respiratory capacity than male islets in both HPAP (p=0.055, Figure 6D) and Humanislets.com (p=0.08, Figure 6H).

We expanded our analysis to include donors of all ages, including donors who lived with T2D (Figure 6I-M). In male islets, T2D was associated with a strong impairment of mitochondrial function. Strikingly, male islets from donors with T2D showed a drastic increase in basal OCR that was not observed in female islets (Figure 6J), with no significant sex difference in maximal respiratory capacity (Figure 6K). In male islets, but not female islets, spare respiratory capacity was also significantly decreased with T2D (Figure 6L), driven by the increase in basal respiration (Figure 6J). Male islets from donors who lived with T2D also demonstrated a significantly impaired ability to increase OCR in response to high glucose; a finding we did not reproduce in female islets (Figure 6M). Thus, our data indicate that T2D is associated with fewer perturbations in islet mitochondrial parameters in females compared with males.

Biological sex did not significantly affect intracellular calcium in whole islets

Intracellular calcium triggers insulin exocytosis [70]. There were no significant differences in intracellular calcium following stimulation with the amino acid mixture, low glucose (3 mM), high glucose (16.7 mM), or KCl (30 mM) in donors without diabetes, regardless of age or diabetes status (Figure S2A-E; S3A-E).

T2D is associated with impaired hormone secretion in response to stimuli in both sexes

Dynamic insulin secretion protocols were different with respect to stimulus and exposure time between all three sites. Among donors without diabetes aged 15-39, no statistically significant sex differences were observed in insulin secretion in either UPenn or Vanderbilt data (Figure

S4A-L). In the same age range, perifusion results from Humanislets.com showed higher insulin secretion from male islets compared to female islets (Figure S4M-Q). Across donors of all ages (Figure 7A-C), T2D was associated with decreased insulin secretion at 0 mM and 3 mM glucose in females but not males in the UPenn perifusion dataset (Figure 7D-E). Amino acid-stimulated insulin secretion was impaired with T2D in both sexes in the UPenn data (Figure 7F). In contrast, Vanderbilt data showed no effect of sex or T2D on insulin secretion at 5.6 mM glucose (Figure 7G), while the Humanislets.com data showed higher insulin secretion at baseline (3 mM glucose) and in 6 mM glucose in islets from male donors without diabetes compared to female donors without diabetes (Figure 7H-I). In all three datasets, T2D was associated with impaired high glucose-stimulated insulin secretion in both sexes (Figure 7J-L). T2D was also associated with impaired IBMX-stimulated secretion in the UPenn and Vanderbilt data (Figure 7M-N). KCl-stimulated insulin secretion was impaired with T2D in both sexes in the UPenn dataset (Figure 7O), males in the Vanderbilt dataset (Figure 7P), and neither sex in the Humanislets.com dataset (Figure 7Q). Hence, T2D was associated with impairment of high glucose-induced insulin secretion in both sexes, and we detected no sex differences that were consistent between the datasets. We note high variability within each dataset, however, reflecting the heterogeneity of nutrient-induced insulin secretion in human islets [44,71,72].

Humanislets.com was the only database with data on insulin secretion in response to leucine and a fatty acid mixture. Consistent with results from the glucose-focused perifusion in this donor pool (Figure S5M-Q), among donors without diabetes, male islets secreted more insulin than female islets at baseline and in response to leucine or fatty acids in both the 15-39 age bracket and across all ages (Figures S5-7). T2D was associated with significantly impaired insulin secretion in response to 5 mM leucine + 6 mM glucose in males; the same trend did not reach statistical significance in females (Figure S6D). T2D was associated with impaired insulin secretion in response to a mixture of oleate and palmitate combined with 6 mM glucose in both sexes (Figure S6I).

Perifusion experiments at UPenn and Vanderbilt also measured glucagon secretion and content. Among donors without diabetes aged 15-39, there were no significant sex differences in glucagon secretion or glucagon content in either dataset (Figure S7). Among donors of all ages (Figure S8A-B), UPenn data showed that T2D was associated with decreased baseline glucagon secretion in female but not male islets (Figure S8C), but there were no sex differences in the Vanderbilt data (Figure S8D). The UPenn data also showed that AAM-stimulated glucagon secretion was decreased in donors who lived with T2D in both sexes, showing a significant reduction in females and a non-significant trend (p=0.09) in males (Figure S8E). In the Vanderbilt dataset, glucagon secretion in response to 1.7 mM glucose and epinephrine was not affected by sex or T2D status (Figure S8F). Neither dataset showed any significant effect of sex or T2D status on IBMX-stimulated glucagon secretion (Figure S8G-H). T2D was associated with decreased KCl-stimulated glucagon secretion in female but not male islets in the UPenn dataset (Figure S8I), and neither sex in the Vanderbilt dataset (Figure S8J). T2D was associated with decreased islet glucagon content in the UPenn data (Figure S8K) but not the Vanderbilt data (Figure S8L). Therefore, glucagon measurements during the perifusion studies at UPenn suggest that T2D is associated with greater abnormalities in alpha-cell glucagon secretion in female islets than male islets, but this was not observed in the Vanderbilt data.

Discussion

The goals of the present study were to (1) identify sex differences in human islet characteristics in the non-T2D state and (2) assess how these characteristics were altered in female and male donors who lived with T2D. By performing integrated analysis of several outcomes and using two independent databases, we discovered robust sex differences across molecular and functional phenotypes in both baseline and T2D contexts. Importantly, T2D was associated with distinct changes in gene and protein expression in female islets compared to male islets. This

suggests sex-dependent mechanisms of pathogenesis, demonstrating the importance of considering biological sex in developing prevention and treatment strategies for T2D.

One key finding from our work was that female islets may have greater mitochondrial activity at baseline and are less susceptible to T2D-associated mitochondrial impairments compared to male islets. Mitochondrial respiration is not only central to GSIS [70,73], but also predicts transplantation success [74–76]. Among young donors without diabetes, mitochondrial pathways were among the top female-biased pathways, consistent with prior studies in humans [35] and rats [77]. Enrichment of mitochondrial pathways may support the higher basal respiration in female islets, which has also been observed in non-human primates [78]. While this higher basal respiration contributed to lower spare respiratory capacity in female islets, this was not associated with defects in insulin secretion. Additionally, although we observed a female-specific decrease in mitochondrial gene and protein expression in islets from donors who lived with T2D, in line with a previous report [35], this did not translate to a functional impairment in mitochondrial respiration in islets from female donors with T2D. A potential explanation for the ability of female islets to maintain function despite changes to mitochondrial gene expression is the higher baseline expression of these genes, providing greater capacity for adaptation, and possibly contributing to the lower risk of T2D [1–4] and later age of diagnosis [79] in women than in men.

Another prominent finding from our analysis was that ribosomal pathways were among the top gene and protein pathways enriched in healthy female islets. A female bias in protein synthesis pathways has been observed in several previous studies in humans [29,33,35], and elevated expression of ribosomal proteins in females has been found across species, including rodent islets [28,29] and most *Drosophila* cells [80]. The female-biased enrichment of pathways related to both protein synthesis and ATP generation may support the energy-intensive process of synthesizing insulin [81]. While our analysis revealed no consistent sex bias in insulin content or insulin secretion to accompany this higher expression of genes related to protein synthesis and

mitochondrial function, this lack of effect may be due to the lower beta-cell proportion in female islets. Further work will also be needed to understand why T2D was associated with an enrichment of cytosolic ribosome genes in male islets. While these pathways were identified as female-biased among young donors without diabetes, and would therefore be expected to promote islet resilience, it is possible that male islets have insufficient mitochondrial activity to support such high levels of protein synthesis.

We further show that alpha-cells may play a greater role in supporting female islets under physiological conditions and may be more perturbed under T2D conditions. Consistent with previous analyses in human islets [42,71], we confirmed that female islets had higher proportions of alpha-cells and lower proportions of beta-cells compared to male islets. The same trend has also been observed in mice [32], in which alpha-cells support beta-cell function by paracrine signaling [82–84]. Under high-fat diet stress, the greater alpha-cell proportion in females may promote beneficial intra-islet structural rearrangements that preserve beta-cell calcium dynamics [32]. Because alpha-cell proportion further increases in female islets during pregnancy, this suggests these cells play an important role during times of additional metabolic stress [85]. An important role for alpha-cells in supporting female metabolic health is further indicated by our finding that T2D-related changes in alpha-cell gene expression were greater females compared to males. Indeed, the HPAP UPenn perifusion data suggested that T2D may be associated with greater changes in alpha-cell function in females than males. Together, these results highlight the importance of characterizing potential sex differences in alpha-cells, which have been understudied in the literature.

Despite multiple sex differences in islet gene and protein expression, and in cellular processes, perifusion data did not demonstrate consistent sex differences in insulin secretion. While prior analyses of earlier iterations of the HPAP islet perifusion data observed increased insulin secretion in female islets compared to male islets [29,33,34], inclusion of new donors shows

the levels of heterogeneity were too high to detect a statistical sex difference. This is consistent with a recent analysis of insulin secretion data from 576 donors from an independent donor pool showing high heterogeneity and no measurable impact of donor sex on insulin secretion patterns[72]. However, humans demonstrate sex differences in insulin secretion *in vivo* [86], acute assays in isolated human donor islets may not capture chronic changes in islet physiology before, during, and after T2D onset. More rigorous investigation of clinical data, with stratification and analysis by sex, will be required to determine how sex differences in islets at the molecular and cellular level contribute to T2D pathogenesis and treatment outcomes. More work will also be needed to capture the effects of genetic ancestry on islet biology. The clinical features of T2D, including BMI at onset [87–92], clinical presentation [93–96], and genetic risk [97–99], can vary widely across geography and genetic ancestry. Human islet characteristics, including insulin secretion [71,100] and gene expression [35] also vary by race. Therefore, while we illustrate sex-specific differences in islet physiology, we also acknowledge that this is only a first step towards improving health equity.

In conclusion, by systematic sex-based analysis of multiple data types, we identified key sex differences in composition, gene expression, and function among donors without diabetes. These differences likely contribute to sex differences in the function of islets from donors who lived with T2D, as well as potentially sex-dependent pathways of T2D onset. Elucidating these sex-dependent disease progression pathways is critical for developing interventions that are effective in both sexes.

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Disclosures

All authors declare no competing interests.

Data availability

Data download sources and dates, code for analysis, and supplementary tables are available at https://github.com/singyoungchen/sex-differences-human-islet-characteristics-.

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Figures

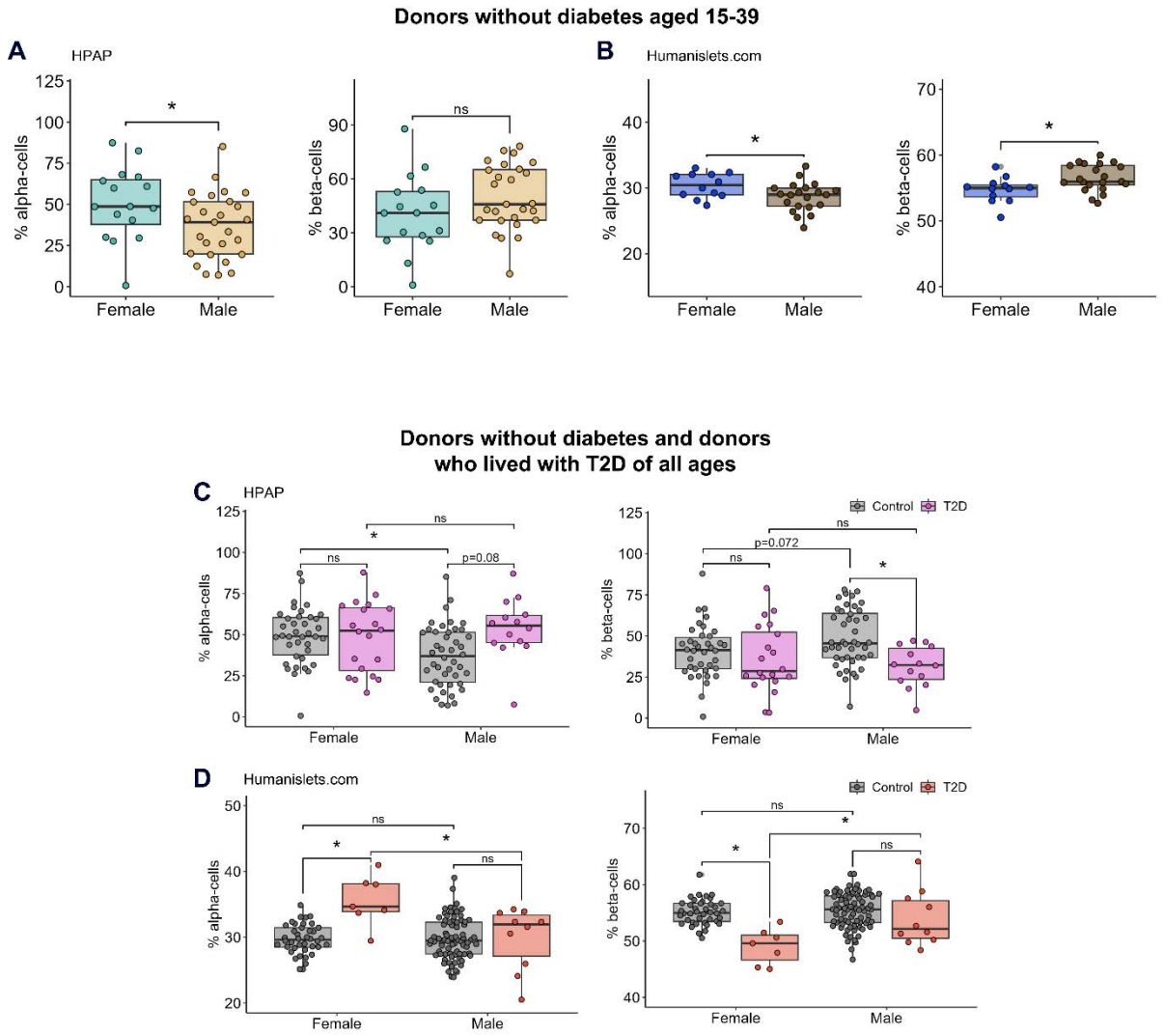

Figure 1. Alpha-cell and beta-cell proportions among total endocrine cells.

Proportions were determined by CyToF in the HPAP dataset (A) or deconvolution from whole islet proteomics in the Humanislets.com dataset (B) in islets from donors without diabetes aged 15-39. Proportions determined by CyToF in the HPAP dataset (C) or deconvolution from whole islet proteomics in the Humanislets.com dataset (D) in islets from donors without diabetes and donors with T2D of all ages.

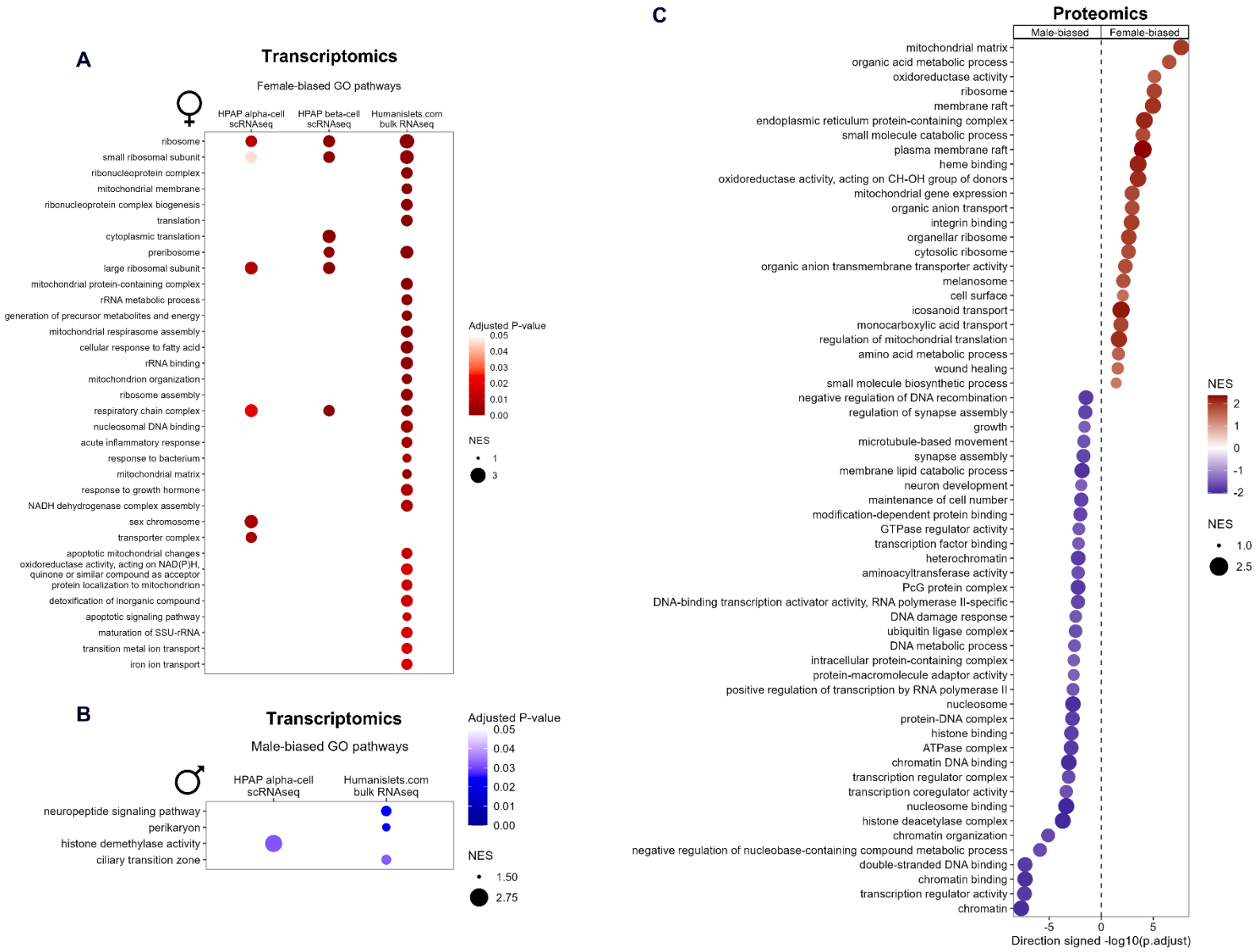

Figure 2. Gene and protein expression analysis of islets from female versus male donors without diabetes aged 15-39.

Top 30 GO pathways that were significantly altered according to sex in GSEA analysis among all transcriptomics datasets (HPAP pseudobulk beta-cell scRNAseq, HPAP pseudobulk alpha-cell scRNAseq, and Humanislets.com bulk islet RNAseq), including (A) female-biased pathways (positively enriched in female) and (B) male-biased pathways (positively enriched in male). Top 60 GO pathways that were significantly altered by sex in GSEA analysis in Humanislets.com proteomics (C). n = 7 female, 17 male for HPAP scRNAseq, 8 female, 18 male for Humanislets.com bulk RNAseq, and 12 female, 21 male for Humanislets.com proteomics. NES: normalized enrichment score.

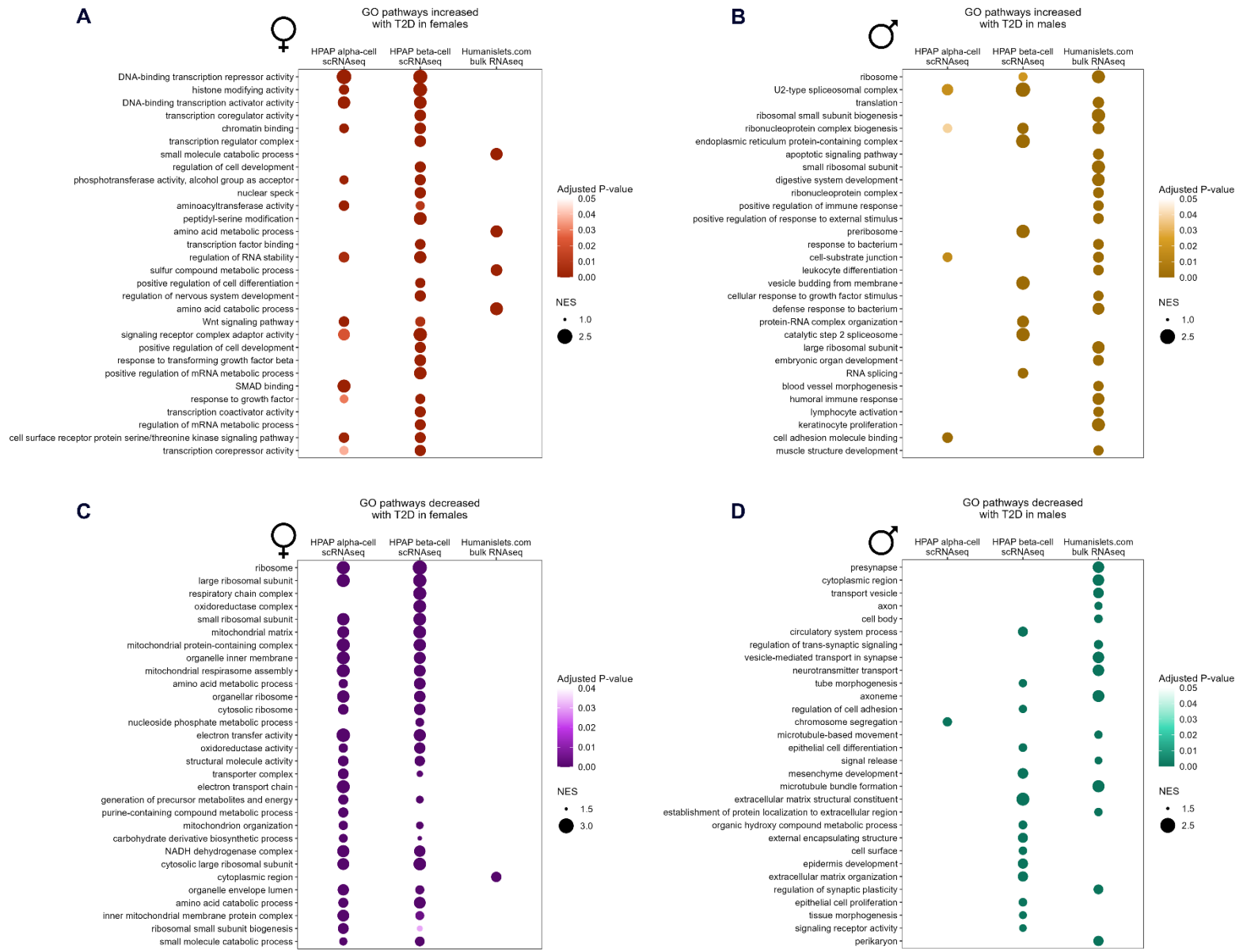

Figure 3. Transcriptomic analysis of islets from donors without diabetes versus donors who lived with T2D in females and

males of all ages.

Transcriptomics results are shown from HPAP pseudobulk beta-cell scRNAseq, HPAP pseudobulk alpha-cell scRNAseq, and Humanislets.com bulk islet RNAseq. Top 30 GO pathways across all datasets that were significantly altered according to T2D status in GSEA analysis, including those increased with T2D in females (A) and males (B) and those decreased with T2D in females (C) and males (D). For HPAP scRNAseq, n = 16 female control, 11 female T2D, 24 male control, and 7 male T2D donors. For Humanislets.com bulk RNAseq, n = 36 female control, 5 female T2D, 66 male control, and 10 male T2D donors. NES: normalized enrichment score.

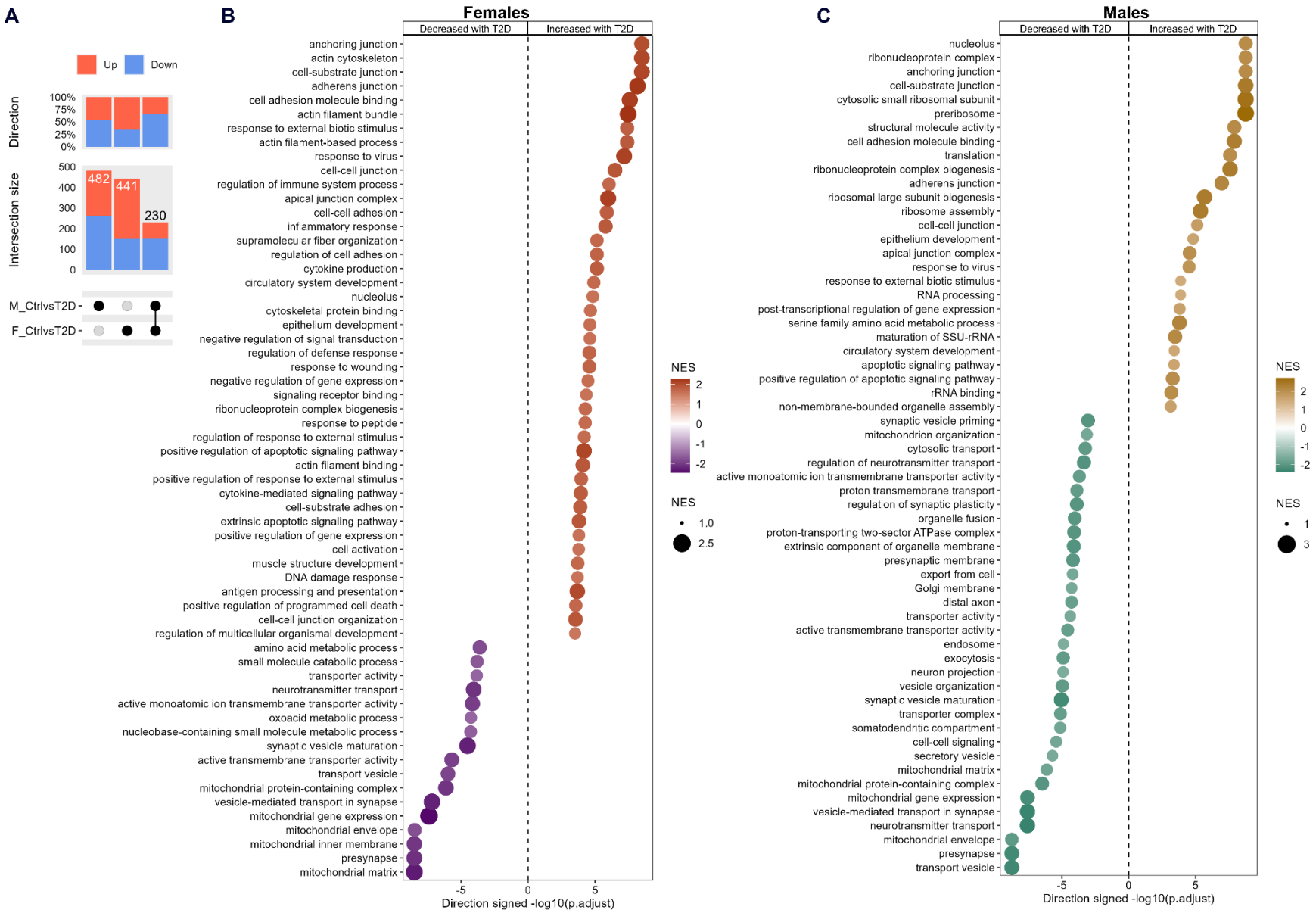

Figure 4. Differential expression and GSEA analysis results comparing the proteomes of islets from donors without

diabetes to donors who lived with T2D in females and males of all ages.

Proteins with significantly altered expression depending on T2D status in male islets, female islets, or both (A). Top 60 GO pathways that were significantly altered according to T2D status in GSEA analysis in females (A) and males (B). n = 40 female control, 7 female T2D, 77 male control, and 10 male T2D donors. NES: normalized enrichment score.

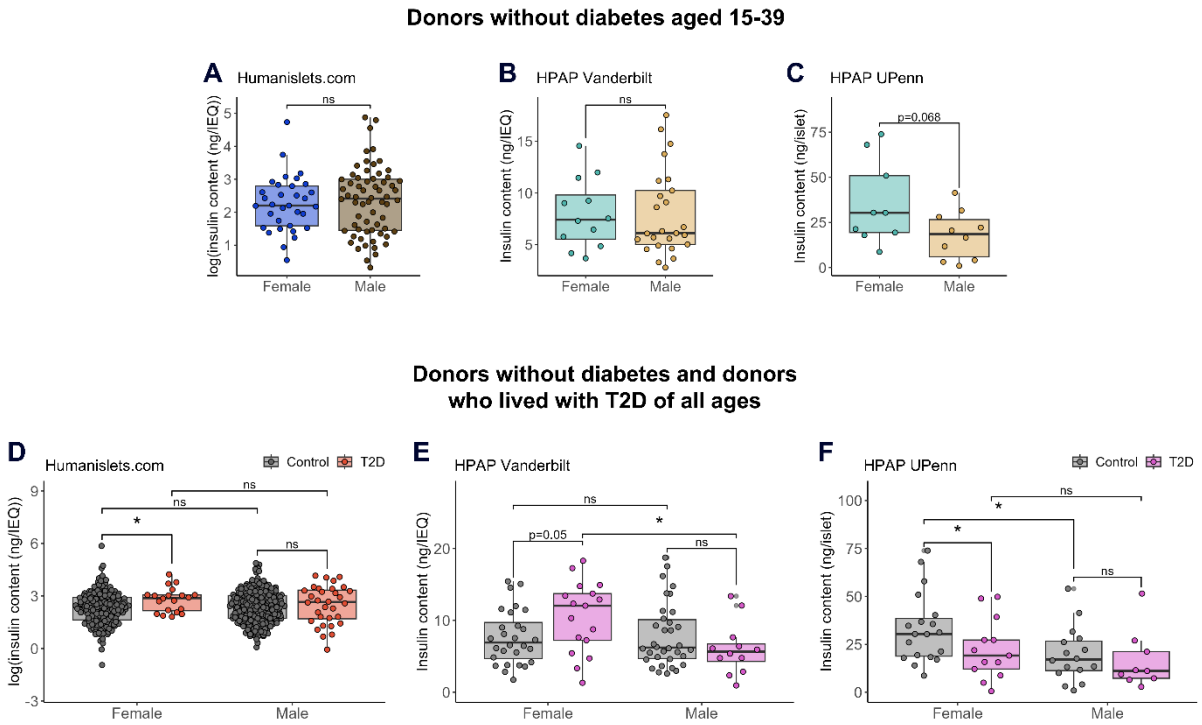

Figure 5. Islet insulin content.

Data shown from Humanislets.com (A), HPAP Vanderbilt (B), and HPAP UPenn (C) for donors without diabetes aged 15-39, and from Humanislets.com (D), HPAP Vanderbilt (E) and HPAP UPenn (F) for donors without diabetes and donors with T2D of all ages.

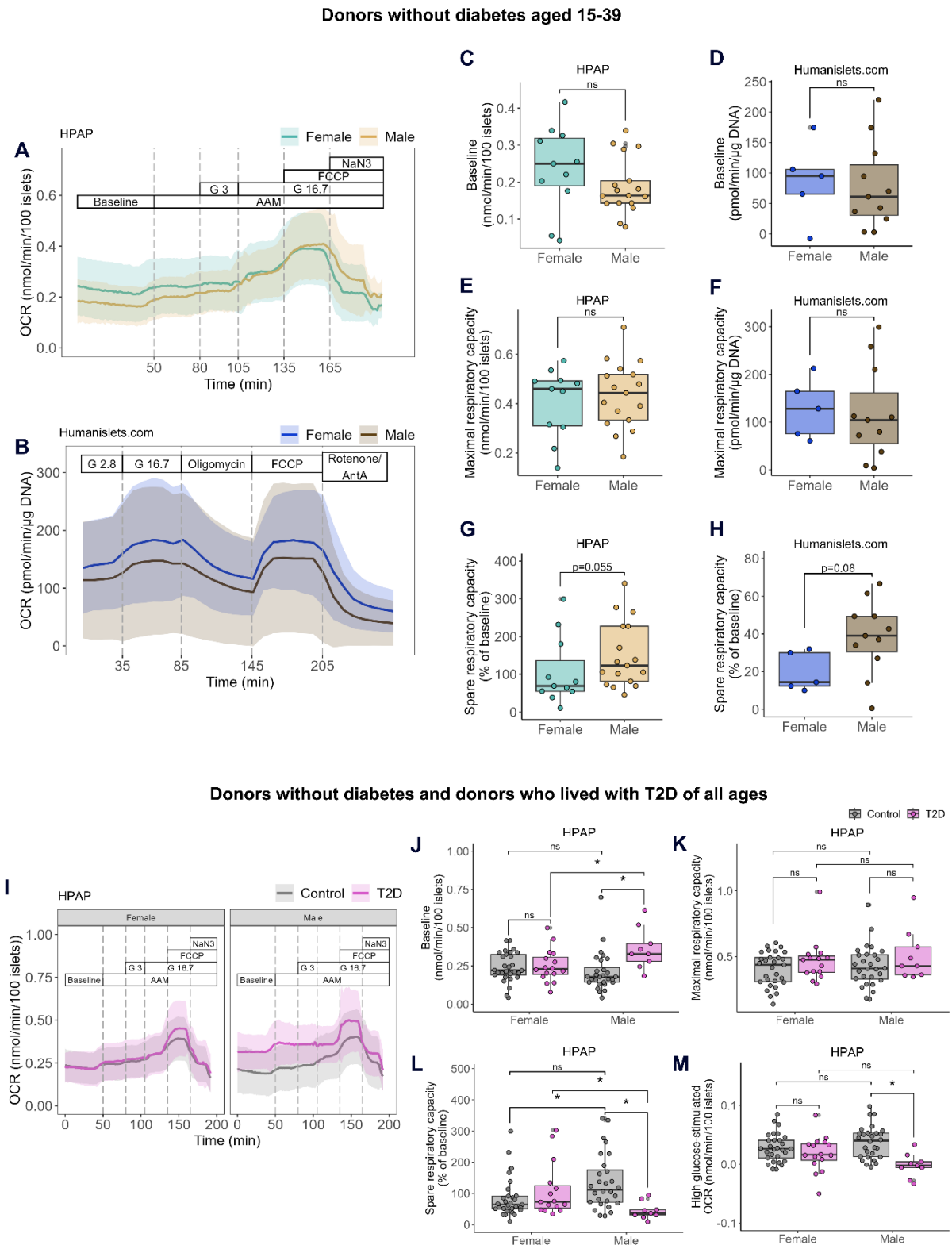

Figure 6. Oxygen consumption rate in whole islets from HPAP and Humanislets.com.

For islets from donors without diabetes aged 15-39, average OCR ± SD tracings from HPAP (A) and Humanislets.com (B). Baseline respiration from HPAP (C) and Humanislets.com (D), maximal respiratory from HPAP (E) and Humanislets.com (F), and spare respiratory capacity from HPAP (G) and Humanislets.com (H) for donors without diabetes aged 15-39. For islets from donors of all ages without diabetes or with T2D, average OCR tracings ± SD for female and male islets from HPAP (I). Summary data for baseline OCR (J), maximal respiratory capacity (K), spare respiratory capacity (L), and high glucose-stimulated OCR (M) from donors of all ages without diabetes or with T2D, from HPAP. AAM = amino acid mix (0.44 mM alanine, 0.19 mM arginine, 0.038 mM aspartate, 0.094 mM citrulline, 0.12 mM glutamate, 0.30 mM glycine, 0.077 mM histidine, 0.094 mM isoleucine, 0.16 mM leucine, 0.37 mM lysine, 0.05 mM methionine, 0.70 mM ornithine, 0.08 mM phenylalanine, 0.35 mM proline, 0.57 mM serine, 0.27 mM threonine, 0.073 mM tryptophan, and 0.20 mM valine, 2 mM glutamine), G 3 = 3 mM glucose, G 16.7 = 16.7 mM glucose, FCCP = carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (mitochondrial uncoupler), NaN3 = sodium azide (cytochrome oxidase inhibitor), AntA = antimycin A (complex III inhibitor).

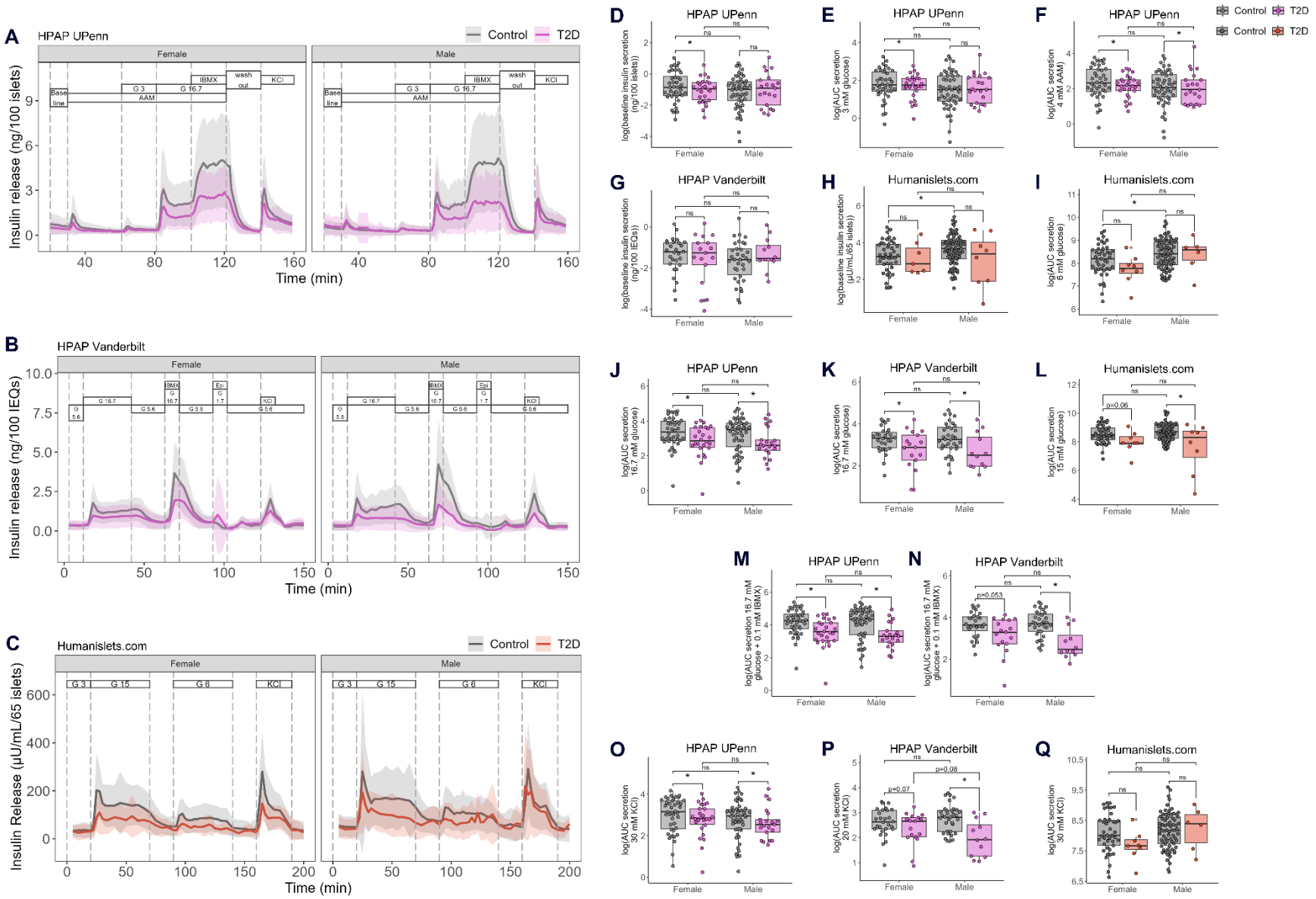

Figure 7. HPAP and Humanislets.com dynamic insulin secretion data following glucose stimulation for islets from donors

without diabetes and donors with T2D of all ages.

Perifusion curves for HPAP UPenn (A), HPAP Vanderbilt (B), and Humanislets.com (C) data. Area under the curve for baseline (0 mM glucose, D), 3 mM glucose-induced (E), and amino acid-induced (F) insulin secretion for HPAP UPenn data. Area under the curve for baseline (5.6 mM glucose) insulin secretion for HPAP Vanderbilt data (G). Area under the curve for baseline (3 mM glucose, H) and 6 mM glucose-induced (I) insulin secretion for Humanislets.com data. Area under the curve for high glucose-induced insulin section for HPAP UPenn (16.7 mM glucose, J), HPAP Vanderbilt (16.7 mM glucose, K), and Humanislets.com (15 mM glucose, L). Area under the curve for high glucose and IBMX-induced insulin secretion for HPAP UPenn (M) and HPAP Vanderbilt (N). Area under the curve for potassium-induced insulin secretion for HPAP UPenn (30 mM KCl, O), HPAP Vanderbilt (20 mM KCl, P), and Humanislets.com (30 mM KCl, Q). AAM = amino acid mix (0.44 mM alanine, 0.19 mM arginine, 0.038 mM aspartate, 0.094 mM citrulline, 0.12 mM glutamate, 0.30 mM glycine, 0.077 mM histidine, 0.094 mM isoleucine, 0.16 mM leucine, 0.37 mM lysine, 0.05 mM methionine, 0.70 mM ornithine, 0.08 mM phenylalanine, 0.35 mM proline, 0.57 mM serine, 0.27 mM threonine, 0.073 mM tryptophan, and 0.20 mM valine, 2 mM glutamine). G = glucose. IBMX = 3-isobutyl-1-methylxanthine at 0.1 mM. Perifusion curves show mean ± SD.

Tables

Table 1. Total genes significantly altered according to biological sex in differential expression analysis among islets from donors without diabetes aged 15-39.

	Number of female-biased genes	Number of male-biased genes
HPAP alpha-cell scRNAseq	5	17
HPAP beta-cell scRNAseq	1	10
Humanislets.com bulk RNAseq	0	0

Table 2. Total genes significantly altered according to T2D status in differential expression analysis for the 3 transcriptomics datasets (HPAP alpha-cell and beta-cell scRNAseq and Humanislets.com bulk RNAseq) in islets from donors of all ages.

	Number of genes increased with T2D in females	Number of genes increased with T2D in males	Number of genes decreased with T2D in females	Number of genes decreased with T2D in males
HPAP alpha-cell scRNAseq	0	0	1	0
HPAP beta-cell scRNAseq	7	8	6	26
Humanislets.com bulk RNAseq	0	375	0	209

Table 3. Total GO pathways significantly altered according to T2D status in GSEA for the 3 transcriptomics datasets (HPAP alpha-cell and beta-cell scRNAseq and Humanislets.com bulk RNAseq) and the Humanislets.com proteomics dataset in islets from donors of all ages.

	Number of GO pathways increased with T2D in females	Number of GO pathways increased with T2D in males	Number of GO pathways decreased with T2D in females	Number of GO pathways decreased with T2D in males
HPAP alpha-cell scRNAseq	90	23	255	52
HPAP beta-cell scRNAseq	251	176	196	379
Humanislets.com bulk RNAseq	54	557	84	178
Humanislets.com bulk proteomics	544	269	225	332