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Review

Review

The Human Islet: Mini-Organ With Mega-Impact

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Abbreviations: ATP, adenosine triphosphate; BMI, body mass index; cAMP, 3′,5′-cyclic adenosine 5′-monophosphate; CFRD, cystic fibrosis–related diabetes; ECM, extracellular matrix; ER, endoplasmic reticulum; ESC, embryonic stem cell; GABA, γ-aminobutyric acid; GCGR, glucagon receptor; GDM, gestational diabetes mellitus; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GPCRs, G protein-coupled receptors; GSIS, glucose-stimulated insulin secretion; GWAS, genome-wide association study; iPSC, induced pluripotent stem cell; K_{ATP}, adenosine triphosphate–sensitive K^+; MODY, maturity-onset diabetes of the young; MPCs, multipotent pancreatic progenitor cells; PP, pancreatic polypeptide; T1D, type 1 diabetes; T2D, type 2 diabetes.

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Abstract

This review focuses on the human pancreatic islet—including its structure, cell composition, development, function, and dysfunction. After providing a historical timeline of key discoveries about human islets over the past century, we describe new research approaches and technologies that are being used to study human islets and how these are providing insight into human islet physiology and pathophysiology. We also describe changes or adaptations in human islets in response to physiologic challenges such as pregnancy, aging, and insulin resistance and discuss islet changes in human diabetes of many forms. We outline current and future interventions being developed to protect, restore, or replace human islets. The review also highlights unresolved questions about human islets and proposes areas where additional research on human islets is needed.

Key Words: islet, β cell, insulin, α cell, glucagon, diabetes, glucose

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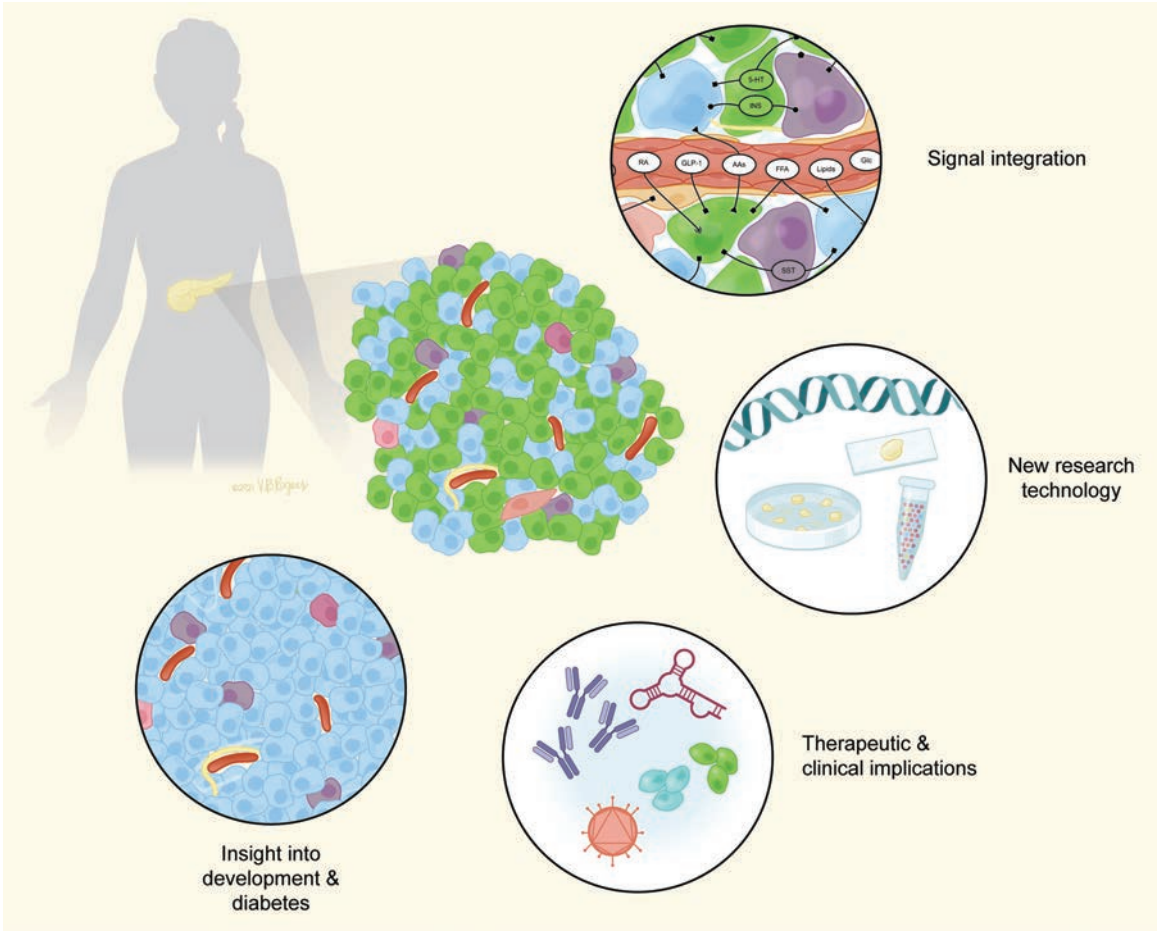
Graphical Abstract



ESSENTIAL POINTS

1. Our understanding of islet biology has advanced over 3 broad eras of scientific discovery.
2. Specific cellular identity markers are being defined for the diverse cell types within the islet microenvironment.
3. Model systems to study human islets are evolving rapidly, allowing mechanistic and multi-"omic" studies of physiology.
4. The human islet is a coordinated mini-organ that integrates systemic and local signals to precisely control blood glucose.
5. Normal human islet physiology is dynamic and influenced by underlying genetics, developmental processes, metabolic state, and cellular progressions during aging.
6. Changes in the human islet during the many forms of diabetes are being studied but are still incompletely defined.
7. Increased knowledge of human islet biology is leading to rational design of exciting new therapies to treat diabetes.

In this review, as part of a series commemorating the centennial of insulin's discovery, we focus on the human pancreatic islet, including its structure, cell composition, development, function, and dysfunction. We highlight recent advances in the understanding of human islet biology that are providing insight into several forms of human diabetes. We also describe how much of the new information about human islet biology is the result of increased availability of human islets for research and the application of new research approaches and technologies to studies of isolated human islets and human pancreatic specimens. First, this review briefly summarizes the evolution of the human islet research over the past 100 years, the events and key decisions that led to

the increased human islet research, and current and future technologies that are advancing the study of human islets and pancreas. The review then discusses our current understanding of human islet function and biology and how islet structure or function becomes perturbed in certain forms of diabetes. Finally, this review outlines future interventions that might prevent or reverse diabetes-associated islet dysfunction or loss and highlights areas where additional research is needed to fill important gaps in our knowledge.

By focusing on the human islet, we do not ignore or minimize important discoveries and research on the physiology or pathophysiology of nonhuman islets. Such studies continue to be essential in advancing our knowledge of islet biology, providing the foundation for investigating these processes in human tissue. In assembling this review, we recognized that many features of human islet biology are incompletely defined, and many of the elegant and detailed observations of molecular pathways and processes in nonhuman islets have been only partially studied, or have not been studied at all, in the human pancreas or human islets. Because of these caveats, we focus our comments primarily on what *has* been examined in human islets and pancreas. Where there are obvious gaps, we highlight knowledge based on studies of nonhuman islets and point to studies needed in the future.

Furthermore, studies of the human islets or human pancreas have inherent limitations, including 1) variability between human specimens that may be the result of age or genetic differences between human donors or how the human specimens were acquired or processed for study; 2) the inability to control the physiology or pathophysiology of human donors prior to pancreas procurement; 3) changes in the human pancreas that occur rapidly after death and possibly changes in human islets during islet isolation or culture; 4) the inability to safely biopsy or sample the pancreas in living individuals (except during surgical procedures); and 5) the cross-sectional nature all studies of human islets or the pancreas, making it more challenging to reach mechanistic conclusions. While acknowledging these important caveats about human islet research, it has become clear that studies of human islets from normal and diabetic donors are required for a complete understanding of the islet's role in different forms of human diabetes. Moreover, there are important differences between human and nonhuman pancreatic islets, including islet cell composition and arrangement, proliferation rates, regulation and expression of certain key genes, etc. In this review, after a brief discussion of islet biology prior to 1921, we will focus on what we have learned about the human islet in the 100 years after the discovery of insulin.

Historical Commentary

Timeline of Human Islet Research

Paul Langerhans, reporting his work in Virchow's laboratory at the Berlin Pathologic Institute in his 1869 medical student thesis, "Contributions to the Microscopic Anatomy of the Pancreas," described in the rabbit pancreas "…cells…gathered in rounded masses, 0.12-0.24 mm in diameter, distributed at regular intervals in the parenchyma…" (1, 2) Laguesse, the French histologist, noted in 1893 a similar cellular arrangement in the human pancreas and referred to these as "ilots de Langerhans" and proposed they might have a role in internal secretions (3). However, the function of this collection of cells remained unknown. The role of the pancreas in glucose homeostasis was established through a series of studies in the 1880s and 1890s by several investigators, including von Mering and Minkowski (4) and later it became evident that the islet was responsible. Two predominant cell types in the islet (α and β) were identified by Lane (5), and changes in islet morphology were first reported by several investigators (6-8) in the first decade of the 20th century, all prior to insulin's discovery in 1921.

Over the last century, diabetes and insulin research has been prominent in advancing scientific methods such as protein and DNA sequencing and cloning of complementary DNA and genes. In reviewing studies of human islets over this time period, one notes 3 broad eras of scientific discovery and key events that have advanced our understanding of human islet biology (Fig. 1). During the first phase, islet morphology and cell identity were predominantly defined by histochemistry with various dyes or chemicals allowing one to distinguish islet cell types (5-7, 12, 20). While bioactive molecules (insulin, glucagon, somatostatin, etc) were isolated from pancreatic digests during this period, conclusive proof of the source of these peptides awaited new advances in cell identification or cell isolation. During the mid-20th century phase, antibodies directed at hormones allowed immunofluorescence studies and the development of the radioimmunoassay, rapidly advancing our knowledge of islet biology (17, 21-24). Discoveries related to islet granule morphology (electron microscopy) and hormone biosynthesis (eg, prohormone processing) began to demonstrate the exquisite nature of islet cells and their ability to synthesize and secrete hormones in response to a variety of physiologic stimuli (25-27). These advances in approaches to study islets coincided with a burst of clinical investigation of insulin secretion in humans, powered by the development of radioimmunoassays for insulin and glucagon and the ability to model glucose usage and production (28-30). The most recent era in islet research began with the cloning of the genes for islet hormones in the

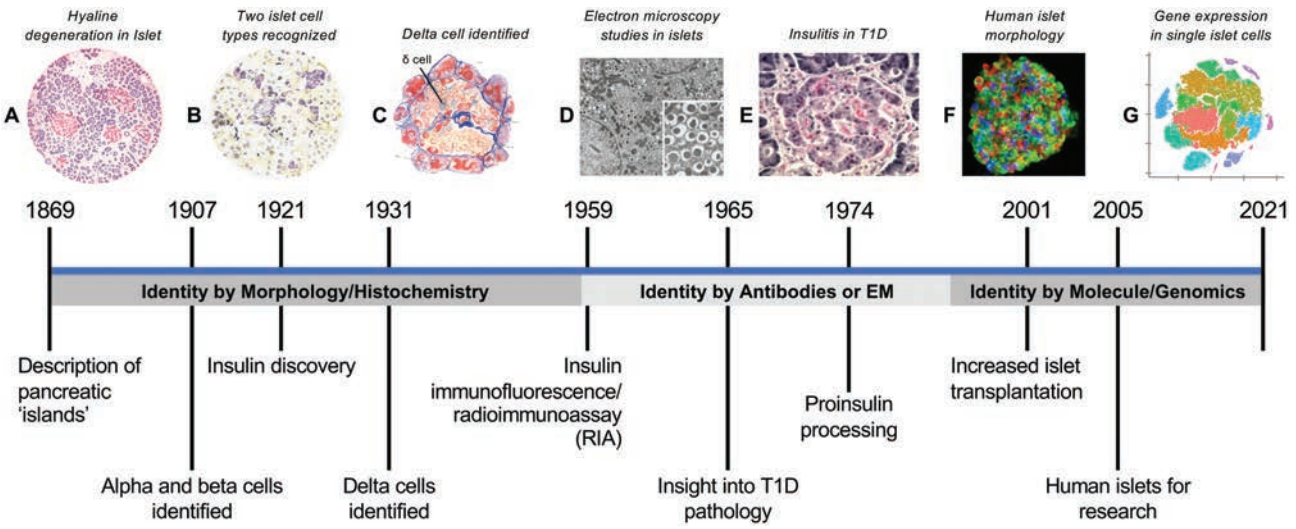


Figure 1. Timeline of key events, discoveries, and technologies related to human islet biology. The upper portion of the figure shows examples of data from the study of human pancreas, islets, or islet cells (described later). The middle portion of the figure shows a timeline of experimental approaches broadly categorized into 3 eras of discovery: 1) identity of islets or islet cells by morphological and histological features; 2) identity of islets or islet cells by antibodies (immunostaining) or ultrastructural features (electron microscopy); and 3) identity of islets or islet cells by genomic or transcriptional profiling leading to molecular signatures. The lower portion of the figure indicates the approximate timing of some important discoveries or events in the understanding of islet biology in each era. These discoveries are illustrated by images or data in the upper panel from some of the original publications positioned at the approximate time of discovery. These selected images and references do not recognize the important work by many scientists because of space limitations. Each image panel in the upper portion of the figure is denoted with a letter: A, A hand-drawn image shows hyaline degeneration in a human islet (likely representing amyloid deposition) in some forms of adult-onset diabetes (9). Differential stainings distinguish endocrine (red in the image) and exocrine (purple in image) areas of the pancreas section. Republished with permission of Rockefeller University Press, from "The Relation of Diabetes Mellitus to Lesions of the Pancreas, Hyaline Degeneration of the Islands of Langerhans," Opie, *The Journal of Experimental Medicine* 5, 1901 (8); permission conveyed through Copyright Clearance Center, Inc. B, A hand-drawn image of a guinea pig islet showing 2 distinct islet cell types noted by differential staining with gentian violet and orange G following fixation with alcohol (referred to as α cells; purple cells in figure) or an aqueous chrome-sublimate (referred to as β cells; light orange cells in panel) (10). Republished with permission of John Wiley and Sons, from "The cytological characters of the areas of Langerhans," Lane, *American Journal of Anatomy* 7, 1907 (5); permission conveyed through Copyright Clearance Center, Inc. C, A hand-drawn image of a human islet showing δ cells (light blue cells noted with black line) identified by Mallory-azan staining (11), republished with permission of John Wiley and Sons, from "A new type of granular. Cell in the islets of Langerhans of man," Bloom, *Anatomical Record* 49, 1931 (12); permission conveyed through Copyright Clearance Center, Inc. D, Electron microscope image of a β cell showing insulin secretory granules in the figure inset (13), reprinted by permission from Springer *Nature: Diabetologia*, "A portrait of the pancreatic B-Cell," Orci, 1974 (14); permission conveyed through Copyright Clearance Center, Inc. E, Lymphocytes in a human islet in type 1 diabetes (15), reprinted from *The American Journal of Medicine* 70, Gepts and Lecompte, "The pancreatic islets in diabetes," p. 111, © 1981 by authors (16), with permission from Elsevier. Gepts originally described the presence of immune cells in an earlier publication (17). F, Confocal microscopy of an isolated human islet showing immunostaining for insulin (green), glucagon (red), and somatostatin (blue) and highlighting the difference in cell arrangement in human islets compared to rodent islets (18), Brissova, Fowler, Nicholson, et al, *Journal of Histochemistry & Cytochemistry* 35, p. 11, copyright © 2005 by authors (19); reprinted by permission of SAGE Publications, Inc. G, Panel illustrates the result of transcriptional profiling of single cells with human islets by sequencing the messenger RNA (mRNA) in an individual cell (single-cell RNA sequencing; more information in Table 4) followed by analysis and projection on a T-distributed stochastic neighbor embedding (t-SNE) plot. Colors correspond to cell clusters grouped by patterns of gene expression. Panel provided by review authors (unpublished).

1980s, that was followed by advances in the genetics/genomics of islet biology and continues with a current focus on characterization of islet cells at the single-cell level (31-33). As discussed later, progress in this era has been greatly accelerated by a dramatic increase in the number and quality of human pancreata and human islets for research.

Increased Availability of Human Pancreas and Islets for Research

While studies with rodent islets were well under way in the early 1970s, thanks to the isolation procedure established by Lacy and Kostianovsky (34), access to human islet material was hampered by logistics of organ procurement and technical challenges of the islet isolation process that releases pancreatic islet "mini-organs" from the vast exocrine tissue (Fig. 2). Building on the work of others (35-37), Ricordi and colleagues in 1988 developed an "automated method" for pancreas dissociation that substantially improved both the quality and quantity of pancreatic islets (38, 39). This improvement in human islet isolation outcomes stimulated a series of early pilot clinical trials of islet transplantation in order to restore euglycemia in individuals with type 1 diabetes (T1D). Unfortunately, most of them resulted in the early loss of graft function and short-lasting insulin independence (39, 40).

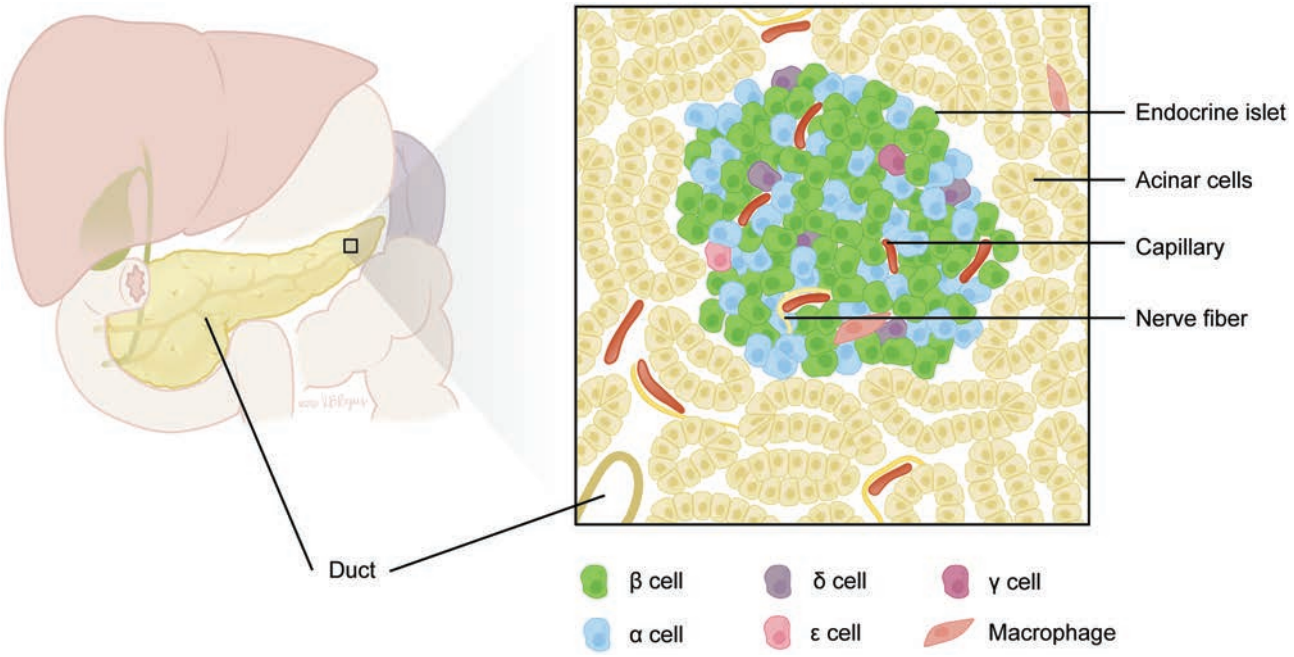


Figure 2. The pancreatic endocrine islet is a mini-organ that coordinates glucose homeostasis. The pancreas, which is broadly divided into head, body, and tail regions, lies behind the stomach in back of the abdominal cavity, with the head positioned in the curve of the duodenum and the tail extending toward the spleen. Most of the pancreatic mass is exocrine tissue, encompassing clusters of digestive enzyme-secreting cells arranged in acini that feed into a branched ductal system joining the common bile duct for secretion into the small intestine. Variations in cystic duct anatomy exist but the most common anatomy is shown here. Blood flow from the pancreas feeds into the portal vein and flows directly to the liver. Endocrine islets are dispersed throughout the gland; they are composed of α, β, δ, γ, and ε cells and also contain capillaries, nerve fibers, and resident immune cells (shown here: macrophages). Text labels refer to examples of anatomic and cellular features; both pancreatic duct and capillary in inset are schematized to show lumen but are lined by ductal epithelium and vascular endothelium, respectively. © 2021 Victoria B. Rogers.

In 2000, the islet transplantation landscape changed dramatically when Shapiro and colleagues reported their results from 7 T1D patients receiving an islet transplant along with a glucocorticoid-free immunosuppressive regimen, which became known as the Edmonton protocol (41). Remarkably, all patients in this and then a larger follow-up clinical study achieved insulin independence (41, 42), with 80% of them remaining insulin-free at 1 year after transplantation (42). This initial success reinvigorated the field of islet transplantation and subsequently led to the international clinical trial of Edmonton protocol (43) and later to the phase 3 clinical trial in North America (44). These and follow-up studies of the Edmonton protocol conducted in more than 800 T1D patients showed that although the rate and duration of insulin independence was relatively limited, islet transplantation was able to ameliorate severe hypoglycemia and improve hemoglobin A_{1c} levels compared to the pretransplantation period (45-49). As a result of these trials, islet transplantation is now offered in Canada, Australia, Switzerland, Italy, France, Scandinavia, and the United States for selected patients with unstable T1D marked by hypoglycemia unawareness and severe hypoglycemic episodes (50-52). Islet autotransplantation has also become more common and effective in individuals undergoing total pancreatectomy for chronic pancreatitis (49, 53-55).

The 2000 report by Shapiro and colleagues (41) represents an important landmark not only for clinical islet transplantation, but also for basic research—the increased islet production for clinical transplantation made human islets accessible to basic science investigators through distribution networks that emerged in North America, Europe, Australia, and Japan as part of clinical islet isolation centers. Notably, the Islet Cell Resource Center Consortium, formed in 2001 and supported by the National Institutes of Health through the National Center for Research Resources, the National Institute of Diabetes and Digestive and Kidney Diseases, and the JDRF, was the first and largest organized effort to provide human islets to researchers in the United States (56). The sustained supply of human islets for basic research over the past 20 years through the Integrated Islet Distribution Program (https://iidp.coh.org/) and its predecessor, the Islet Cell Resource, IsletCore at the University of Alberta (http://www.isletcore.ca/), Nordic Islet Network (https://nordicislets.medscinet.com/en.aspx), and other islet isolation resources (57-59) created new scientific opportunities to move studies of islet biology beyond cell lines and rodent model systems to better understand

mechanisms of human disease with programs such as the Innovative Medicines Initiative for Diabetes (https://www.imi.europa.eu/projects-results/project-factsheets/imidia), T2DSystems Consortium in Europe (https://www.t2dsystems.eu/t2dsystems), the Human Islet Research Network (https://hirnetwork.org/), and Accelerating Medicines Partnership Type 2 Diabetes (https://fnih.org/our-programs/AMP/accelerating-medicines-partnership-type-2-diabetes-project). While methodological and experimental challenges remain (60-64), scientific advances in human islet research further motivated initiatives focused on comprehensive phenotyping of pancreas and islets from individuals with T1D such as Network for Pancreatic Organ donors with Diabetes (https://www.jdrfnpod.org/), and more recently the Human Pancreas Analysis Program (https://hpap.pmacs.upenn.edu/) (65). Studies highlighted in the present review emphasize the value and importance of human pancreas and islets for basic research to accelerate our understanding of human islet biology and design of new and transformative therapies for diabetes.

New Experimental Approaches to Study Human Pancreas and Islets

With the increased availability of human islets and human pancreas for research in the past decade or so, scientists have developed a plethora of systems (Fig. 3 and Table 1) to probe various components of the islet to understand its function in the context of endogenous or external stimuli. Several of these techniques were initially developed using rodent islets and can be applied to rodent and nonhuman primate samples in addition to human tissue. Early islet research relied heavily on histology and morphological characterization, as illustrated by prominent papers defining pancreatic tissue architecture and cellular ultrastructure (14, 105-107). Since that time, imaging technologies have progressed rapidly and now encompass a diverse array of techniques that allow biologists to push experimental boundaries and pose complex questions at increasing spatial and temporal resolution (Table 2). New tissue-clearing techniques such as Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining/in situ-hybridization-compatible Tissue hYdrogel (CLARITY) enable 3-dimensional reconstruction for greater appreciation of the islet microenvironment, and with multiplexed imaging, large numbers of antigens can be visualized at once, bringing high-dimensional data to imaging (see Fig. 4 for examples of 3-dimensional reconstructions and multiplexed imaging of the human pancreas). Finally, the advent of in vivo imaging—both of human islets transplanted into the anterior chamber of the eye of immunodeficient mice, as well as the recording of

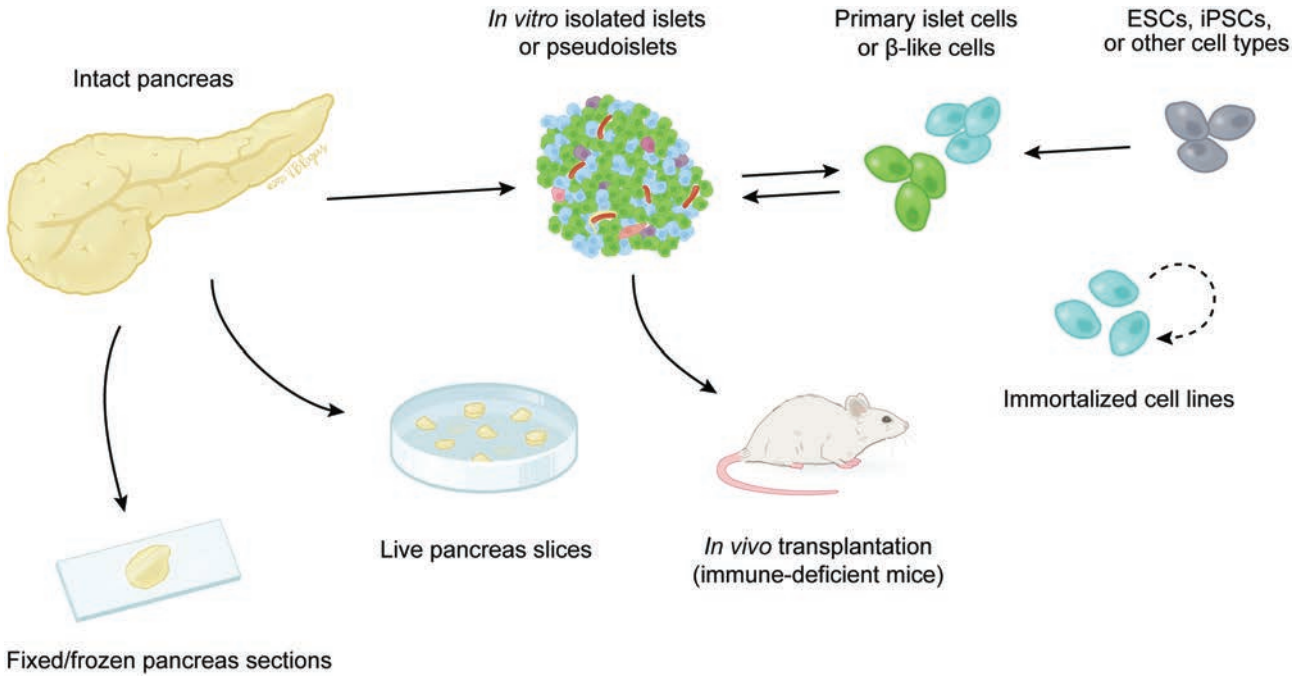


Figure 3. Models used to study the human pancreatic islet. Using a cadaveric donor organ, islets can be isolated from surrounding exocrine tissue or can be dispersed further into single cells. Additionally, pancreatic sections can be fixed and/or frozen for histological analysis or processed into "slices" to perform experiments ex vivo. As an alternative to primary tissue, β-like cells can be generated from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or other cell types, and immortalized β cell lines are also available. Cells from multiple sources can be (re)combined to form pseudoislets, and native islets or pseudoislets may also be transplanted into immune-deficient mice for in vivo physiological analysis. See Table 1 for detailed information about these model systems. © 2021 Victoria B. Rogers.

Table 1. Model systems for studying human islets

Model	Description	Applications and advantages	Limitations	Key references
Histological sections	Pancreas processed and sections mounted onto slides for imaging	• Reflective of endogenous pancreas • Can be processed in multiple ways for several techniques	• Static snapshots • No ability to manipulate tissue • Study of a small pancreas region	Used by many laboratories
Pancreas slices	Small intact sections of living pancreatic tissue with exocrine and endocrine structure preserved	• Preserves microenvironment architecture • Avoids islet isolation process • Allows for study of interaction between endocrine and exocrine compartments	• Islet/pancreas blood flow and innervation are not maintained • Material is limited • Culture duration is limited	(66-69)
Isolated islets in vitro	Endocrine pancreas (islets) enzymatically and physically separated from exocrine pancreas for study	• Enriches for endocrine compartment of pancreas • Islet structure relatively intact • Can be processed in multiple ways for several techniques	• Heterogeneity of human islets (variable islet size in single preparation and variable purity between preparations) • Islet vascularization and innervation are not maintained • Limited supply; unpredictable availability • Culture duration is limited	(64, 70) Review: (60)
In vivo transplantation (mice)	Human islets engrafted into immunodeficient mice	• Islets can be studied in a dynamic in vivo environment • Permits longer-term studies • ACE site allows for in vivo imaging of graft	• Hormones from endogenous mouse islet cells may affect studies • Glycemic set point of mouse is different from that of humans	(71, 72) ACE: (73-75) Reviews: (76, 77)
Pseudoislets	3D organoids that combine islet cell populations to generate structures resembling native islets	• Allows for efficient genetic manipulation and even distribution of molecules (e.g., virus) to all cells • Composition (proportions of cell types within islet) can be manipulated prior to reaggregation • Uniformity of resulting pseudoislets (composition and size)	• Potential effects of initially breaking cell-cell connections • Not clear how closely pseudoislets resemble primary islets • Variability between pseudoislet formation techniques/protocols • See also: limitations for isolated islets	(78-84) Review: (85)
SC-derived insulin-producing cells and other islet cells	In vitro differentiation of undifferentiated ESCs or iPSCs into β-like or other islet cells	• Controlled source of cells for study • Allows for development-related questions • iPSCs can be individualized	• Not clear how closely SC-derived islet cells resemble primary islet cells • Heterogeneity between protocols, reagents, and cells produced • ESC source ≠ iPSC	β cells: (86-91); α cells: (92) Applications: (93-95) Reviews: (96-101)
Immortalized cell lines	Examples: EndoC; immortalized human β cell line derived from fetal pancreatic tissue	• Amenable to manipulation (gene knockdown, chemical treatment, CRISPR, etc) • Single-cell line reduces heterogeneity between experiments • Some versions allow for cell growth to be arrested	• Not clear how closely cell lines resemble primary islet cells • Difficult to propagate/culture	(102-104)

Abbreviations: 3D, 3-dimensional; ACE, anterior chamber of the eye; CRISPR, clustered regularly interspaced short palindromic repeats; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; SC, stem cell.

Table 2. Techniques for static imaging of human islets

Technique	Description	Advantages	Limitations	Key references
Traditional IHC or IF	Antibodies used to visualize antigens (markers); primary or secondary antibodies linked to an enzyme or a fluorescent dye	• Simple workflow • Flexibility to co-stain with markers of choice in different subsets	• Limited number of fluorophores or chromogens per sample • Limited by antibody species specificity (in some cases) • Dependent on quality and specificity of antibody	Used by many laboratories
3D imaging (including tissue clearing)	Multiple 2D images are captured at different tissue depths to enable reconstruction of the 3D structure	• Better resolution (depth) is superior to widefield • Ideal for 3D imaging and surface profiling • Tissue remains intact	• Increased resolution at cost of decreased signal intensity • Many traditional IHC antibodies do not work on cleared tissue	(108-111) Review: (112) See also: Fig. 4A
Multiplexed imaging	Antibodies conjugated to heavy metals and resolved by IMS or conjugated to oligonucleotide barcodes and imaged in iterative cycles (co-detection by indexing; CODEX)	• Visualize 30+ antigens on a single tissue section at submicron resolution • Highly quantitative • CODEX: preserves tissue; can co-register with other chromogens	• Requires specialized (conjugated) antibodies • IMC: tissue destroyed in process • IMC: limited imaging area • CODEX: time-intensive imaging • Evolving technologies	IMC: (113, 114) CODEX: (115) Reviews: (116, 117) See also: Fig. 4B and 4C
Imaging mass spectrometry	Ionization of tissue at x/y coordinates to generate mass spectra; used to visualize the spatial distribution of biomarkers, metabolites, peptides, or proteins	• Detect thousands of analytes across a sample surface • Label-free • Provides both localization (spatial) information and relative abundance • Can detect posttranslational modifications	• Protein ID can be challenging • Specialized preparation of tissue and slides • Limited spatial resolution compared to other techniques	(118, 119) Review: (120) See also: Fig. 4D
ISH and FISH	Labeled probes of complementary nucleic acid oligomers detect specific RNA molecules	• Specific with good design of oligomers • Can be applied to archival materials and frozen tissues • Can be combined with IHC to detect protein as well as mRNA of interest	• mRNA degradation often occurs in the pancreas	(33, 121) Review: (122)
EM	Beam of accelerated electrons used to produce an image based on how electrons interact with sample	• Higher resolving power than light microscopy • Can detect subcellular structures (eg, organelles) at very high resolution	• Biological specimens require specialized preparation for stabilization and ultrathin sectioning • Potential artifacts from sample preparation or charging/conductance	(123-126) Reviews: (127, 128) See also: Fig. 4E

Abbreviations: 2D, 2-dimensional; 3D, 3-dimensional; CODEX, co-detection by indexing; EM, electron microscopy; ESC, embryonic stem cell; FISH, fluorescent in situ hybridization; ID, identification; IF, immunofluorescence; IHC, immunohistochemistry; IMS, imaging mass spectrometry; ISH, in situ hybridization; mRNA, messenger RNA; SC, stem cell.

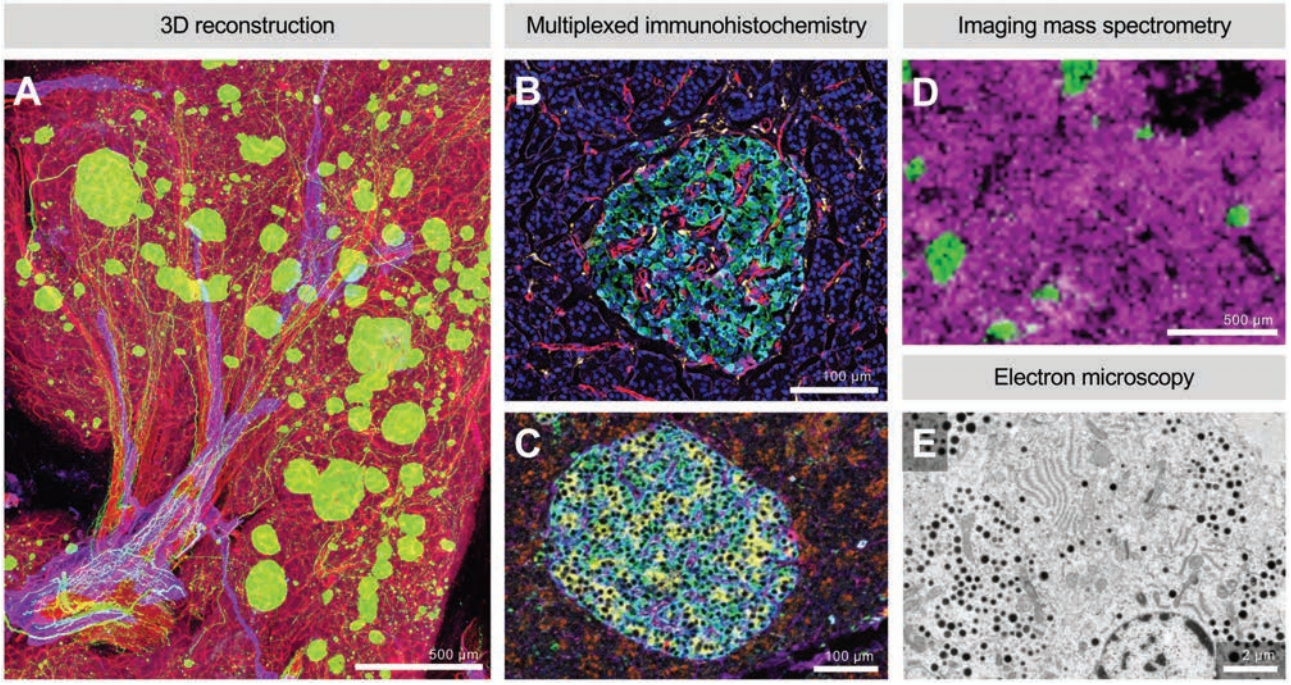


Figure 4. Common cellular imaging techniques used to study human islets. A, Cleared tissue imaged for 3-dimensional reconstruction is instrumental to understanding intricate structures such as nerves and vasculature. Labeling of the human pancreas for PGP9.5 (islets and nerves; green), CD31 (blood vessels; red), and D2-40 (lymphatic vessels; blue). Image provided by Shiue-Cheng Tang, PhD (National Tsing Hua University, Hsinchu, Taiwan) and is related to reference (108). B and C, Multiplexed immunohistochemistry provides information of overall tissue architecture, cell identity, and cell heterogeneity, accomplished by using antibodies conjugated to oligonucleotide barcodes (B, co-detection by indexing, CODEX; or C, metal isotopes; imaging mass cytometry. B shows an islet labeled for C-peptide (β cells; green), glucagon (α cells; cyan), somatostatin (δ cells; magenta), CD31 (capillaries; red), IBA1 (macrophages; yellow), collagen IV (extracellular matrix; purple), and DAPI (4′ 6-diamidino-2-phenylindole; dark blue); image provided by review authors (unpublished). C shows an islet labeled for C-peptide (β cells; chartreuse), glucagon (α cells; cyan), somatostatin (δ cells; light blue), pancreatic polypeptide (γ cells; red), CD8 (medium blue), CD56 (light orange), CD68 (green), collagen (purple), and nuclear factor κB (dark orange). Image from the Human Pancreas Analysis Program (hpap.pmacs.upenn.edu) (65, 129). D, Imaging mass spectrometry maps spatial distribution of proteins and metabolites. Panel is from a 20-µm image of human pancreas analyzed by matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS); *m/z* 703.6 (green) is likely sphingomyelin and *m/z* 758.6 (magenta) is likely phosphatidylcholine (34:2). Image provided by Boone M. Prentice, PhD (University of Florida, Gainesville, Florida, USA), and is related to reference (119). E, To probe islet ultrastructure, electron microscopy provides enhanced resolution that highlights intracellular components with macromolecular resolution. Image from Nanotomy database (www.nanotomy.org/OA/nPOD), associated with references (124, 130).

labeled molecules applied to living human tissue slices—represent an exciting opportunity to transform findings from cross-sectional, "frozen in time" studies to a more detailed understanding of the mechanisms governing human islet growth and function (66, 74).

In addition to imaging, islet biologists have also adapted emerging physiological techniques to measure cellular respiration and metabolism, electrophysiological activity, and hormone secretion in isolated human islets or single cells (Table 3). Over the past several years, there has been an effort to standardize the functional assessment of human islets distributed for research in the United States, which resulted in the formation of the Human Islet Phenotyping Program (https://iidp.coh.org/) of the Integrated Islet Distribution Program (64). Among other assays, the Human Islet Phenotyping Program conducts a dynamic perifusion to help investigators understand how each islet preparation responds to various stimuli, ideally with this assessment being used by study authors to inform the interpretation of experimental results (60). Finally, the advent of "omics" approaches (particularly those with single-cell resolution) has generated considerable interest in characterizing the epigenome, transcriptome, proteome, and metabolome of human islet cells (Table 4). In fact, many currently referenced "markers" of human endocrine cell subpopulations were generated from the first studies to apply single-cell RNA sequencing to human islets (31-33). Follow-up studies are needed to confirm the presence—and activity—of corresponding proteins. Our current understanding of islet structure and function draws from many of the aforementioned models and techniques, often integrating tools across platforms to confirm findings. Acknowledging the inability to cite all published efforts and techniques, a summary of our current knowledge is relayed in the following section.

Table 3. Techniques to assess human islet physiology

Technique	Description	Advantages	Limitations	Key references
Static incubation	Hormone secretion by islets incubated in given secretagogue	• Adaptable to large screens • Does not require specialized equipment	• Poor/no temporal resolution of hormone secretion	Used by many laboratories
Perifusion	Temporal resolution of hormone secretion in response to dynamic delivery of secretagogues	• Time resolution (can appreciate initiation of hormone secretion and shutting off secretion) • Flexible system can assess multiple different secretagogues • Can scale up or down with microfluidic optimization	• Native innervation and vasculature not maintained • Difficult to appreciate cell or islet heterogeneity	(70, 131-133)
Electrophysiology	Measurement of the electrical properties of islet cells	• Detailed cellular physiology of ion flow and exocytosis • Excellent time resolution • Can isolate individual channels and/or ion currents	• Measurement performed on a single cell may not be representative • Relatively low throughput	(134-136)
Ca^{2+} dynamics	Use of fluorescent calcium indicators to track intracellular calcium dynamics in islet cells	• Excellent time resolution • Allows spatial resolution of individual cells within islet	• Difficulty to deliver calcium dye throughout the entire islet or to specific cell types • Ca^{2+} dynamics do not always match hormone secretion	(137-140)
Mitochondrial metabolism	Measurement of oxygen consumption rate to assess mitochondrial function	• Assessment of subcellular organelle function • Measurement of cellular energy metabolism	• Difficult to assess changes in specific cell types due to studying a heterogenous mixture of cells • Does not always match hormone secretion	(140-144)
Secretagogue stimulation in humans	Assessment of hormone secretion and glucose metabolism in the whole organism by various secretagogue delivery routes (oral, bolus, or infusion)	• Reflects actual human physiology • In vivo measurement	• Does not isolate islet function; no sense of islet or cell heterogeneity • Difficult to isolate specific factors • Hormones are assessed in peripheral circulation after passing through liver • Hormone clearance and uptake may affect conclusions	(145-150)

The Human Islet: A Master of Signal Integration

Human Islet Structure

The human islet, a vascularized and innervated mini-organ, consists primarily of endocrine cells: α cells, which secrete glucagon; β cells, which secrete insulin; δ cells, which secrete somatostatin; pancreatic polypeptide (PP) or γ cells, which secrete PP; and ε cells, which secrete ghrelin (Fig. 2). The islet also contains capillaries (endothelial cells and pericytes), neuronal projections, resident immune cells, and fibroblasts (Figs. 2, 5). The contributions of nonendocrine cells to overall islet function have become increasingly appreciated, though many in vitro studies still

Table 4. Techniques to investigate human islet "omics"

Description and methods		Advantages	Limitations	Key references
Epigenomics	Heritable chemical or physical changes in chromatin • **DNA methylation** (eg, bisulfite sequencing, WGBS; ChIP-seq • **Histone modifications** (eg, MS, ChIP-seq) • **Chromatin landscape** (eg, assay for transposase-accessible chromatin using sequencing; ATAC-seq)	• Can infer longer-term trends than the "snapshot" of transcriptome • WGBS: high coverage, single-base resolution • ChIP-seq: maps specific DNA binding proteins • ATAC-seq: simple prep; high signal-to-noise ratio; requires fewer cells than other techniques	• WGBS: high cost declining; prone to reaction artifacts • ChIP-seq: quality of data relies on antibody quality; more material needed • ATAC-seq: variable efficiency of DNA cleavage • New, under development technologies	(151-155) Reviews, general: (156, 157)
Transcriptomics	Bulk or single-cell RNA-seq, including mRNA, microRNA, lncRNA • Isolation of total RNA (purification/enrichment based on type of RNA to be profiled) • Library prep using reverse transcription to generate cDNA, PCR amplification • Single-end or paired-end sequencing	• Broad, unbiased detection of mRNA transcripts • High resolution to identify splice variants or post transcriptional RNA editing • Bulk sequencing can capture low-abundant transcripts • Single-cell sequencing resolves heterogeneity within cell populations	• Captures a snapshot in time of the total transcripts present in a cell • Does not necessarily reflect protein levels • RNA fragmentation during library prep may introduce bias • Hard to compare data across platforms/techniques	(31-33, 158-161) Reviews, islet: (162-164) Reviews, general: (165)
Proteomics	Large-scale study of proteins, including abundance/turnover and posttranslational modifications • Analytical separation methods include gel purification and MS • **Bottom-up analysis**—protein mixtures subjected to proteolytic cleavage before mass analysis • **Top-down analysis**—intact proteins are ionized and analyzed	• Unbiased broad view of proteins in a quantitative fashion • Flexible system with many modifications available, including various separation techniques, ionization approaches, and mass analyzers • Posttranslational modifications and unknown proteins can be identified	• Number of proteins identified is lower than by transcriptional profiling • Protein ID can be challenging	(166-173) Reviews, islet: (117, 174, 175)
Metabolomics	Broad characterization of substrates and products of metabolism • Wide variety of analytical separation methods to target metabolites • Sample analysis by MS-based approaches or NMR spectroscopy • Isotope labeling	• Directly reflects underlying biochemical activity; integrates both genetic and environmental regulation • MS: unbiased detection of metabolites in a quantitative fashion • NMR: nondestructive (tissues analyzed directly); high reproducibility • Targeted metabolomics can determine an exact concentration of a known metabolite • Isotope labeling enables estimation of metabolic flux	• Reactions take place continuously/dynamically, so analytical techniques reflect only a "snapshot" (specific time under specific conditions); shortest time scale of the "omics" • MS: requires tissue extraction • Metabolite ID can be challenging	(176) Select reviews: (177-179)

Table 4. Continued

Description and methods		Advantages	Limitations	Key references
Integrated "omics"	**Patch-seq:** single-cell electrophysiology measurements in combination with transcriptomic sequencing	• Links transcriptional profile with physiology profiles at a single-cell level • Helps identify how precise changes in gene expression may contribute to functional heterogeneity	• Low throughput • Dispersed cells may differ in physiological responses • Evolving technology	Islets: (180) General: (181, 182)
	CITE-seq: cellular indexing of transcriptomes and epitopes by sequencing; method using oligonucleotide-labeled antibodies	• Integrates cellular protein and transcriptome measurements into a single-cell readout	• Limited by antibody availability • Evolving technology	(183)
	Spatial transcriptomics: nontargeted sequencing in situ; ordered attachment of spatially barcoded oligos that preserves positional information throughout mRNA sequencing process	• Does not require known targets like traditional in situ hybridization techniques	• Limited spatial resolution • Extent of gene capture unclear • Evolving technology	Pancreas: (184, 185) General: (122, 186-188)

Abbreviations: cDNA, complementary DNA; ChIP-seq, chromatin immunoprecipitation sequencing; lncRNA, long noncoding RNA; mRNA, messenger RNA; MS, mass spectrometry; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; RNA-seq, RNA sequencing; SC, stem cell; WGBS, whole-genome bisulfite sequencing.

necessitate a reductionist approach. We provide an overview of human islet cell composition, including highlighting key nonendocrine components, and then specifically look at endocrine cell structure and identity. A recent series of reviews provides greater detail on these topics (189, 190).

Human Islet Composition

In contrast to rodent islets, which contain a β cell–rich "core" and α and δ cells on the periphery, adult human islets display more variability in composition and exhibit more heterotypic contacts between α, β, δ, γ, and ε cells (19, 191, 192). While rodent islets typically consist of 75% to 80% β cells and 15% to 20% α cells, human islets have proportionately fewer β cells (55%-75%) and more α cells (30%-45%) (19, 191-195). Much less abundant are δ and γ cells (representing less than 10% each), with ε cells being particularly rare (estimated to be < 1% of all islet cells) (193); see Fig. 4B and 4C for examples of endocrine cell composition. Human islets range considerably in size (~ 50-500 μm in diameter), with an average of 1500 cells per islet. Variability in endocrine cell ratios between individuals is mirrored by heterogeneity in β cell mass (196, 197), though differences in endocrine cell distribution between pancreatic regions are relatively minor (198). The exception is that γ cells are strikingly abundant in a posterior lobe of the pancreatic head region, referred to as the "uncinate process" (199-202). Quantification of endocrine cell populations has come both from isolated islets and pancreatic sections (19, 113, 193, 195) but contribution of other cell types such as endothelial cells, stromal cells, leukocytes, neuronal elements, and extracellular matrix to islet volume has not been systematically examined. Interestingly, studies suggest that most δ cells are located close to islet capillaries and have an elongated shape as well as filopodia-like processes that increase their potential influence on nonimmediate neighboring cells (203, 204). There is growing evidence of a critical role of somatostatin and δ cells in regulation of islet function in health and disease; somatostatin is a potent paracrine inhibitor both of insulin and glucagon secretion (205). These roles are further discussed in later sections (β Cell, Neurohormonal control: intra-islet signals and α Cell, Neurohormonal control).

Nonendocrine cell populations and extracellular matrix

Nonendocrine cells and the extracellular matrix of the islet likely play important, yet incompletely defined, roles in islet homeostasis—either by delivering nutrients and soluble factors or by providing signals that influence islet cell health and function (206). While rodent islets are highly vascularized, with thick and highly fenestrated capillaries (207-210), human islets have a much lower vascular density (211, 212). Owing to experimental limitations, knowledge of in vivo human islet vascularity and blood flow remains elusive, though a recent report suggests that blood flow may not be unidirectional (111,

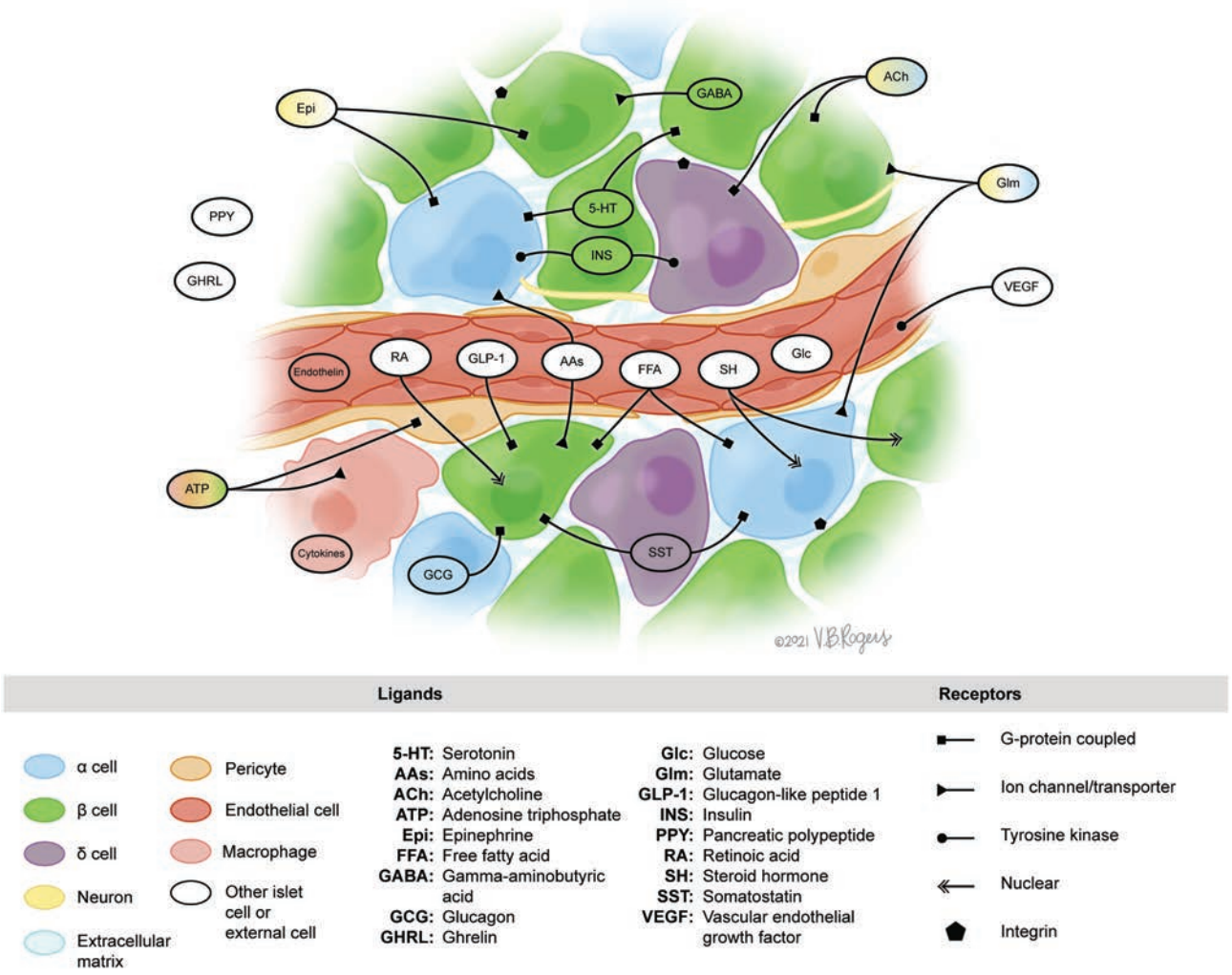


Figure 5. The human islet microenvironment contains a diversity of cells that are intricately connected. Schematic depiction of endocrine cells (β, dark green; α, blue; δ, purple), islet vasculature (red), neuronal processes (yellow), macrophages (pink), and pericytes (pale green). Ligands are colored according to the predominant cell type(s) that produce them or to show they are primarily delivered to the islet via the systemic blood flow; in addition to acting as ligands, some nutrients can also be metabolized (glucose, amino acids [AA], free fatty acids [FFA]). Lines depict local action on islet cells through major receptor categories. Key signaling molecules and receptors are shown; see text for discussion of several of these ligands and their effects on α and β cells. The authors emphasize the complex nature of signaling within the islet microenvironment but note that many pathways are necessarily excluded in this depiction because of space constraints. © 2021 Victoria B. Rogers.

213). Tissue clearing is now revealing more detailed structures of vascular networks; see Fig. 4A. Islet capillary networks are lined with elongated endothelial cells (214-216) and though it is clear pericytes are present, our knowledge of their function in human islets is still evolving (206, 217). Because endothelial cells respond to diverse stimuli such as hypoxia, angiogenic factors, and cytokines, and secrete growth factors, signaling molecules, and basement membrane components, it is likely that such interactions with human islet cells influence their function (218-221). For more detailed information on signaling between vascular cells and endocrine cells, see reviews by Richards, Raines, and Attie (222), Peiris and colleagues (223), and Almaça and colleagues (206).

Neuronal processes and immune cells are found with human pancreatic islets, suggesting that these provide signals influencing islet hormone secretion. For example, neuronal processes project into the human islet from extrapancreatic nerves and may enable modulation by the central nervous system (CNS) (224, 225). Autonomic axons are closely associated with capillaries (226-228), presumably helping to coordinate insulin secretion and other responses to stimuli. Resident immune cells in islets largely belong to T cell and macrophage lineages, with occasional β cells (229-232). Interestingly, these cells tend to be more numerous in peri-islet regions (representing the interface between endocrine and exocrine tissue; see IBA1$^+$ macrophages in Fig. 4B and CD8$^+$ T cells in Fig. 4C) (229, 231). Since islet macrophages are known to play crucial roles in mouse pancreas and islet development, further study of their role in human islet development and in type 2 diabetes (T2D)-associated inflammation will be of great interest (233-235).

The human islet extracellular matrix (ECM) is unique in that human endocrine cells produce a basement membrane that is distinct from the basement membrane of the endothelium and is composed of collagens, heparan sulfate proteoglycans, and laminin (236-239). The islet interstitial matrix consists of collagen, elastin, fibronectin, and various polysaccharides, and together with the basement membrane provides cell support and anchorage (215, 220, 240-242). In addition, it is becoming increasingly appreciated that ECM also influences cell phenotype and function, signaling primarily through integrins but also providing a "sink" for secreted growth factors that interact with unique receptors (220, 240-243). It has also been proposed that the islet ECM provides a polarized microdomain to promote endocrine granule fusion (244, 245). Further investigation is needed specifically into human islets to address these potential functions.

Endocrine Cell Structure

At the ultrastructural level, the common function of all islet endocrine cells is readily apparent: These cells are protein-producing, protein-packaging, and protein-secreting factories, with distinctive secretory granules that also serve as the site of most prohormone processing (see image in Fig. 4E). The β cell has been most extensively studied, catalyzed by the identification of insulin secretory granules in early electron microscopy studies and seminal studies of prohormone processing (insulin, C-peptide, etc) (14, 25, 105, 106, 246). Glucagon-containing secretory granules are similar in size to those of β cells, but their electron-dense cores are distinct, surrounded by a tightly fitted membrane and chromogranins and synaptophysin variably distributed throughout the granule (105, 106, 247). Delta cells contain larger (450-800 nm) secretory granules with lower electron density with synaptophysin and chromogranins present throughout the granule matrix (105, 106, 203, 247). In γ cells, PP is packaged into small, spherical secretory granules that tend to be heterogeneous—some resemble glucagon-containing granules (106, 248). Like γ cells, ε cell ultrastructure resembles that of α cells but its secretory granules tend to be smaller (105, 106).

Endocrine Cell Identity

Beyond distinctive hormones, secretory granules, and subcellular machinery, islet endocrine cells are often defined by their signature of cell surface proteins (eg, receptors, ion channels) or by a network of signaling molecules and transcriptional regulators that define cell identity and function. For example, canonical transcription factors in β cells such as MAFA, NKX6.1, PDX1, PAX6, NKX2-2, ISL1, NEUROD1, FOXO1, and FOXA2 are crucial both for establishing and maintaining β cell identity as well as coordinating the production and secretion of insulin (94, 249-252). All β cells are not the same, as heterogeneity and different β cell subsets have been defined by cell surface markers and islet function (160, 253, 254). Similarly, α cells express factors regulating glucagon production and secretion, notably ARX, IRX1/2, MAFB, PAX6, NKX2-2, ISL1, NEUROD1, and FOXA2 (94, 249, 250, 255). Less is known about regulatory networks in δ, γ, and ε cells, but HHEX is thought to direct δ cell differentiation (256, 257), and γ and ε cells express subsets of transcription factors also found in α cells (249, 258). While there are clear similarities in cellular identity markers between human and nonhuman islets, there are some important differences, most typified by discrepancies in the phenotype of certain forms of monogenic diabetes in mice and humans (255, 259). For example, while mice and humans heterozygous for PDX1 mutations are phenotypically similar (260-263), heterozygous mutations in HNF1α, HNF4α and other maturity-onset diabetes of the young (MODY) transcription factors do not appear to result in similar islet dysfunction in mice compared to that seen in humans (264-266). Moreover, compensatory mechanisms likely differ; for example, while Ngn3-deficient mice do not develop endocrine cells at all, NGN3 loss-of-function mutations in humans produce variable (and less severe) phenotypes (255, 267, 268).

Fortunately, a large amount of data about human islet cell identity markers is rapidly becoming available as single-cell technologies are applied to human islet cells by a number of groups. While not yet completely defined, emerging patterns of gene expression are beginning to define islet cell identity. To categorize genes whose expression may be enriched in or specifically expressed by certain human islet cell types, we aggregated gene lists from several prominent single-cell RNA-sequencing studies (31-33) and display genes with agreement in 2 or more studies in Table 5. Although this list is not exhaustive—and, in many cases, awaits confirmation of protein expression—RNA-sequencing studies provide a helpful framework to begin understanding islet cell-specific signatures. As expected, each cell type is enriched in the transcript for its respective hormone and several known transcription factors, as well as the expected hormone-processing genes *PCSK1* (β cells) and *PCSK2* (α cells). Expression of ion-sensing complexes (*KCNK16* and *SLC6A6* in β cells) and amino acid transporters (*SLC38A4* and *SLC7A2* in α cells) emphasizes the environment-sensing function of endocrine islet cells. Furthermore, δ cells and γ cells express transporters known to recognize specific neurotransmitters (*SLC17A6* and *SLC6A4* for glutamate and serotonin, respectively), consistent with the presence of receptors for adenosine triphosphate (ATP), cholecystokinin B, and acetylcholine already documented in human δ cells (271-273).

In the case of α and β cells, gene lists can be relatively easily cross-referenced to complementary experiments on

Table 5. Transcripts significantly enriched in each human endocrine subtype, as determined by scRNA-seq[a]

	α	β	δ	γ	ε
Hormones, secreted factors	*CRH, GCG*	*ADCYAP1, BMP5, IAPP, IGF2, INS*	*AQP3, FFAR4, GABRB3, LEPR, MS4A8, SLC17A6, UNC5B*	*ABCC9, CHRM3, FGFR1, NPFFR2, SLC6A4*	*ASGR1*
Receptors, membrane transport	*DPP4, FXYD3, FXYD5, FXYD6, KCNJ6, LAPTM4B, SDC2, SLC22A17, SLC38A4, SLC40A1, SLC7A2*	*CASR, KCNK16, ROBO1, ROBO2, SLC6A6*	*CD9, PARVB, SERPINA1*	*CPB1, PCDH10, SCGB2A1, SPOCK1, THSD7A*	
Basement membrane/ ECM/ adhesion-related	*CD99L2, FAP, MUC13, NPNT, PAPPA2, SMOC1, SPOCK3, TM4SF4*	*TFF3, TIMP2, PVRL3*	*EHF, HHEX, NCOA7, PSIP1*	*ETV1, FOXP2, ID4, MAF, MEIS2, PAX6*	*SERPINA1, TM4SF5*
Transcription factors or regulators	*ARX, CBX6, HMGB3, IRX2, MAFB*	*BHLHE41, CDKN1A, CDKN1C, EIF4A2, HOPX, ID1, MAFA, NKX6-1, PDX1, RPB4, SAMD11*	*AKAP12, RBP4, TPPP3*	*AKAP9, TUBB2A*	
Motility, scaffold, transport	*CLU, COTL1, GC, KCTD12, PALLD*	*FAM159B, GSN, SYNE2*	*PRG4*	*PDK4*	
Metabolism or lipid-related	*ETFDH, FABP5, HIGD1A, PPP1R1A, PLCE1, PLIN3, SPTSSB*	*ERO1LB, G6PC2, HADH, SCD5*	*BCHE, RGS2, SEC11C*	*CHN2, DPYSL3*	*ACSL1*
Miscellaneous enzymes	*CHID1, F10, FSTL5, GLS, GPX3, LOXL4, PCSK2, RGS4, SERPINE2, TTR*	*ENTPD3, GPX2, PCSK1, PFKFB2, PRSS23, RRAGD, RASD1, RGS16*	*LINC00643, LY6H, UCP2*	*CMTM8, FGD4, PXK, SERTM1, STMN2*	
Other	*ARRDC4, CFC1, CRYBA2, FAM84A, GADD45G, NUCB1, TMEM176A, TMEM176B*	*ASB9, DLK1, LMO1, MEG3, MT1F, NPTX2, SCGN, SYT13*			*ANXA13, PHGR1*

Abbreviations: ECM, extracellular matrix.

[a]Selected genes from lists reported by ≥2 individual studies: references (31-33, 269); (270) for ε cells only. Please note that sequencing and analysis methods differed among studies and thus the list is not exhaustive (n = 4 studies queried for α, β, δ, and γ cells; n = 3 for ε cells).

protein expression or function, but in the less common endocrine cell subtypes, transcriptional signatures are more difficult to validate as those cells have not been as robustly studied. Nonetheless, human δ cell-enriched transcripts include the known transcription factor *HHEX*, as well as receptors for free fatty acids (*FFAR4*) and leptin (*LEPR*). Similarly, human γ cells are enriched for *CHRM3*, a muscarinic receptor. Knowledge of an ε cell signature remains limited because of the low frequency of ε cells even in large single-cell sequencing data sets, but the expression of the ECM-related genes *SERPINA1* and *TM4SF5* hint at a role in local surveillance and cell-cell communication. Further exploration into these unique endocrine cells is on the horizon and will add clarity and define signatures of islet cell identity.

Looking Forward: Topics to Explore Relating to Human Islet Composition and Endocrine Cell Identity

- What is responsible for the heterogeneity of islet cell populations, are these populations stable, and do distinctive islet cell subsets have a role in islet adaptation or diabetes?

- What is the vascularization state and direction of blood flow in human islets?
- What role do endothelial cells, pericytes, macrophages, and other nonendocrine cells within the human islet mini-organ play in nutrient sensing, hormone secretion, and the response to challenges such as puberty, pregnancy, and insulin resistance?

Human Islet Physiology and Function

Within the islet, α and β cells integrate systemic signals circulating in the blood with locally released signals derived from the islet microenvironment (274). We provide a broad overview of the major nutrient and neurohormonal signals as they relate to control of insulin and glucagon secretion, as other endocrine cell hormones are thought to primarily act locally with little direct contribution to whole-body metabolism. Thus, we discuss somatostatin from δ cells in the context of how it regulates secretion from β and α cells. In the case of PP and ghrelin, little is currently known about the role of these hormones in the human islet. Finally, this overview is not exhaustive, with the complex nature of the islet suggesting that other signals likely also have a role in modulating insulin or glucagon secretion. An important caveat is that most pathways and signals regulating α and β cell function that we will discuss have been defined in studies of nonhuman islet cells with some not yet confirmed in human islet cells.

β Cell

Nutrient control: glucose, amino acids, and lipids

Glucose-stimulated insulin secretion (GSIS) involves the coordinated relay of metabolic, electrical, and chemical signals within the β cell (135, 274-279). One unique aspect of GSIS in human β cells is that the facilitated diffusion of glucose occurs via transporter GLUT1, in contrast to GLUT2 in rodent β cells (280-282). Most subsequent steps in this glucose-triggering pathway are thought to be similar in nonhuman and human islets, including processing by glucokinase, glucose metabolism by glycolysis in the cytoplasm and the tricarboxylic acid cycle in the mitochondria, an increase in ATP:adenosine diphosphate ratio, closure of the ATP-sensitive K^+ (K_{ATP}) channels, rise in intracellular Ca^{2+}, and insulin granule exocytosis (283, 284). A representative insulin secretory profile from islet perifusion and a schematic of β cell signaling with the components of the GSIS pathway that the secretagogues within the perifusion target is shown in Fig. 6A and 6B.

Glucose metabolism also has a potentiating effect on insulin secretion by other stimuli that depolarize the human β cell via the so-called amplifying pathway (285-288), which is downstream from the intracellular Ca^{2+} increase and thought to be mediated by mitochondrial-derived metabolic coupling factors such as guanosine triphosphate, isocitrate, or NADPH (276, 289-293). Thus, mitochondrial metabolism is crucial both for the generation of ATP as well as the intersection of various metabolic pathways and the regulation of these coupling factors (141, 294-296). The triggering and amplifying pathways help to create the characteristic biphasic insulin secretory response seen in vitro with an abrupt increase in glucose (Fig. 6A). These pathways have been discussed in detail in excellent recent reviews by Rorsman and Ashcroft (284) as well as by Campbell and Newgard (288).

While glucose is the primary physiological regulator of insulin secretion, circulating amino acids such as arginine, leucine, alanine, glutamine, and glycine (26, 297-299), metabolites such as glutamate (300), and lipids (301) can also influence insulin secretion. For amino acids, this effect may be mediated by transport and metabolism, through binding to extracellular receptors, or via direct depolarization of the plasma membrane (302). Glutamate, an excitatory neurotransmitter, may signal through ionotropic or metabotropic glutamate receptors to influence insulin secretion, though the significance of these pathways in human β cells is not as well understood (303, 304). Importantly, amino acids may also influence insulin secretion indirectly, particularly through the α cell (305). In addition to intracellular lipid metabolism, extracellular fatty acids can signal though G protein-coupled receptors, the most well studied being GPR40 (FFAR1).

Neurohormonal control: incretins and epinephrine

Other neurohormonal signals, most notably the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), modulate insulin secretion. Receptors for GLP-1 and GIP are G_s-coupled GPCRs that primarily signal by activating adenylyl cyclase to increase 3′,5′-cyclic adenosine 5′-monophosphate (cAMP) (Fig. 6A and 6B) (306-310). Signaling from GLP-1 and GIP alone is not sufficient to stimulate insulin secretion but acts synergistically to potentiate GSIS (311-313). Epinephrine, a sympathetic hormone primarily from the adrenal glands or released locally by sympathetic nerves, acts to raise blood glucose and prevent hypoglycemia in part by shutting off insulin secretion. Epinephrine is a ligand for multiple receptors, but human β cells primarily express α_2-adrenergic receptors, G_i-coupled GPCRs that signal by inhibiting adenylyl cyclase to reduce cAMP and by activating G protein–coupled inwardly rectifying potassium channels (314, 315).

Neurohormonal control: intra-islet signals

While systemic signals are crucial in the control of insulin secretion, the structure of the islet creates a unique microenvironment for local intra-islet signals (274, 277, 316). There are many secreted factors from the various

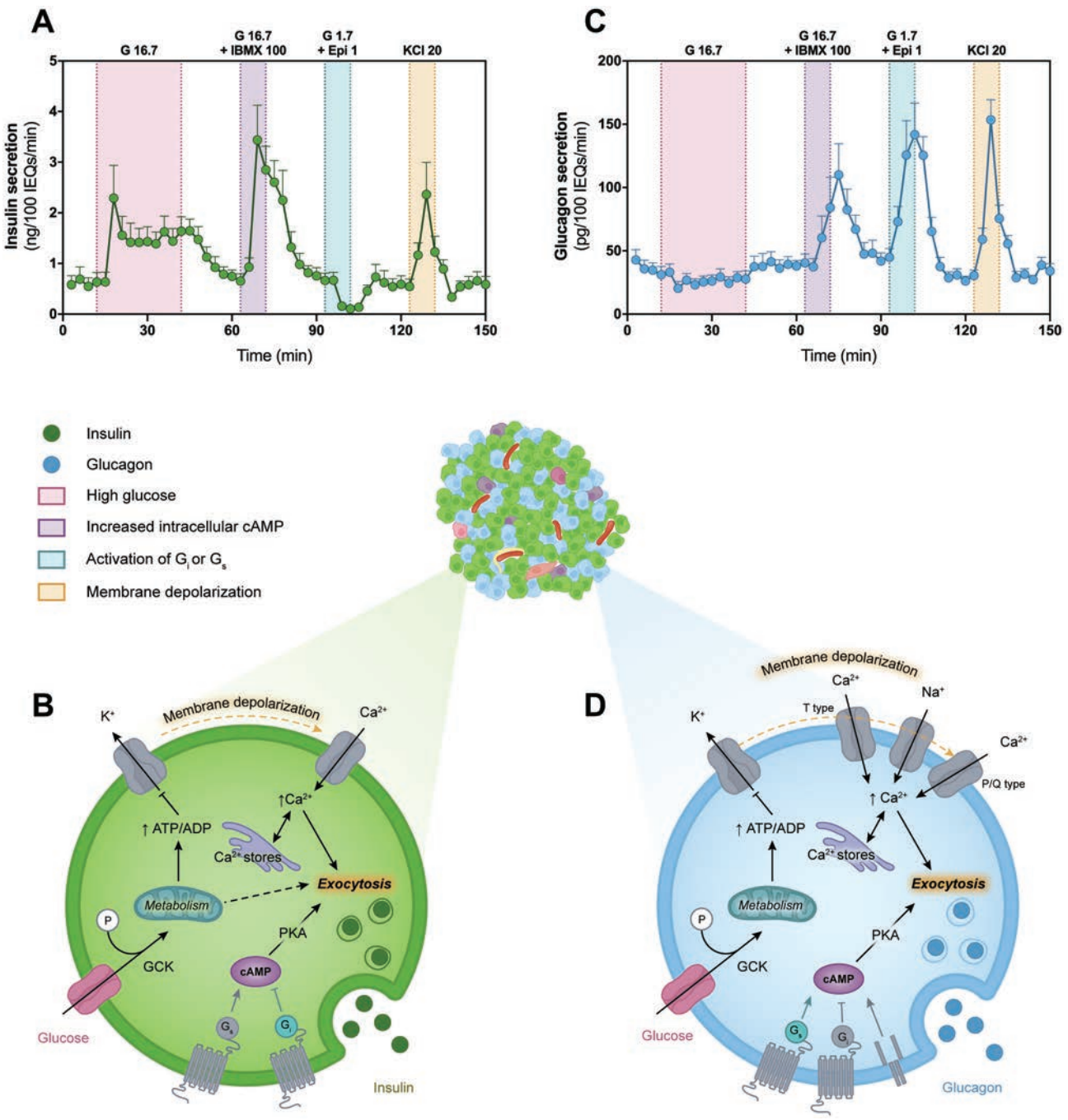


Figure 6. Intracellular mechanisms controlling insulin and glucagon secretion from β and α cells. Perifusion traces depict endocrine cell function and associated schematics of insulin secretion from A and B, β cells, and C and D, glucagon secretion from α cells. Exposure to high glucose (pink), a 3′,5′-cyclic adenosine 5′-monophosphate (cAMP)-potentiator (IBMX; purple), low glucose and epinephrine (teal), and direct depolarization (KCl; orange) represent the standardized protocol used by the Human Pancreas Phenotyping Program (HIPP) to evaluate human islet preparations distributed through IIDP and the Alberta IsletCore; traces shown are from 7 nondiabetic donors, ages 17 to 49 years, analyzed through HPAP (hpap.pmacs.upenn.edu). Schematics of the B, β cell, and D, α cell, highlight major signaling pathways controlling hormone secretion; in the α cell these pathways are less well defined and so the pathways shown are presumptive. Key components within the cell are color coordinated with the corresponding perifusion stimuli to conceptualize how the intracellular pathways result in the secretion dynamics shown in A and C.

cells within the islet that can often act on numerous receptors and cell types, thus in this review we highlight a selection of the most well-studied factors (Fig. 5). These paracrine signals, which have been shown to be active in human islets but have been mechanistically studied primarily in nonhuman islets, allow for an additional layer of β cell control. Indeed, individual β cells do not display the same coordinated secretion pattern seen in intact islets; paracrine signals from α cells are crucial in establishing species-specific glycemic set points (278, 317). Further, β

cells within an islet synchronize their electrical and Ca^{2+} responses through gap junctional coupling. Importantly, this coupling is crucial to a robust insulin secretory response, as individual β cells do not respond in the same fashion as an intact islet (27, 318). Recent work has demonstrated that within islets, β cells may take on different roles—some may be fine-tuned to be more sensitive to glucose and act as a pacemaker or "hub" β cell in the islet (139, 319). These studies, which were performed in mice, are supported by mathematical modeling of human islets (253, 320) but require more work to clearly establish this concept in human islets. Further, the means by which these hubs cells may transmit signals remains debated (321-323).

Despite the opposing physiological actions of insulin and glucagon, glucagon can regulate and potentiate insulin secretion (324). The glucagon receptor (GCGR) is expressed by β cells and is a G_s-coupled GPCR acting through cAMP (325). The GCGR has a high degree of sequence homology with GLP-1R (326) and several groups have demonstrated that the ligands to these receptors, glucagon and GLP-1, respectively, are capable of activating either receptor (305, 327, 328). Physiologically, this is thought to predominantly manifest as α cell–derived glucagon signaling through both GLP-1R and GCGR on the β cell; however, as GLP-1 is also derived from proglucagon, GLP-1 produced within the islet may contribute to islet signaling (329, 330). This foundational α to β cell communication within the islet also sets up scenarios where nutrient signaling to the β cell, such as from amino acids, can come indirectly through the α cell (305).

It is becoming clear that δ cells provide important local inhibition to β cells. The regulation of δ cells is not as well understood, but somatostatin secretion increases with glucose in a dose-dependent manner and involves calcium-induced calcium release (275, 331, 332). Somatostatin secretion may also be stimulated by local signals, including the peptide urocortin3 released from β cells or by ghrelin from ε cells (333, 334). Somatostatin signals to the β cell through 1 of 5 SSTR isoforms, with SSTR2 thought to be the most prominent in humans (335, 336). All isoforms are G_i-coupled GPCRs that signal similarly to the α_2-adrenergic receptor discussed earlier. Thus, under physiologic conditions, while somatostatin provides inhibitory feedback to modulate and possibly prevent the oversecretion of insulin, it does not completely block insulin secretion (275, 337). Recent reviews have discussed the δ cell and provided more details on its potential signaling pathways (205, 275).

Other signals derived in the islet that modulate hormone secretion include ghrelin, extracellular ATP, serotonin, γ-aminobutyric acid (GABA), and acetylcholine. Ghrelin is secreted primarily from cells of the gastric mucosa and would enter islets in the circulation, but it can also be secreted locally in the islet by ε cells. It is most well known for its role as an appetite stimulant but also acts to inhibit insulin secretion via the growth hormone secretatgogue receptor, which is G_i-coupled in β cells (338-342). ATP is stored in insulin granules of the β cell and can be co-secreted with insulin or secreted by "kiss-and-run" exocytosis where dense insulin cores are retained within the granule (343, 344). Human β cells primarily express ionotropic purinergic receptors, thus setting up an autocrine-feedback network (345, 346). These receptors are permeable to Na^+, K^+, and Ca^{2+}, and thus when activated depolarize the cell and increase insulin secretion. Serotonin, a monoamine neurotransmitter, is produced by the β cell and co-secreted from insulin granules (347). Serotonin signaling is particularly important during pregnancy, when its increased production mediates islet adaptations to metabolic demands (348, 349). GABA, the major inhibitory neurotransmitter in the CNS, is derived from glutamate via glutamic acid decarboxylase and is synthesized in β cells at some of the highest levels outside the CNS (350). While small amounts of GABA may be released with insulin granules, most GABA is secreted independently of glucose from the cytosol of β cells via an alternative pulsatile secretory pathway (351). Human β cells express the ionotropic $GABA_A$ receptors, which are permeable to Cl^- when activated (352). Acetylcholine is the major neurotransmitter of parasympathetic nerves; however, parasympathetic innervation is relatively sparse in the islet and thus the major local source of acetylcholine is likely the α cell (227, 353). Acetylcholine signaling is complex, with multiple receptors at play, though the G_q-coupled M3 receptor is thought to be the primary cholinergic receptor in β cells (273).

α Cell

Nutrient control: glucose, amino acids, and lipids

In comparison to glucose-stimulated insulin secretion, the molecular mechanism by which glucose regulates glucagon secretion is far less clear, with multiple, often contradictory, hypotheses presented and no single model explaining all the dynamics of glucagon release (354-359). Furthermore, even less is known about human α cells; thus, much of glucagon secretion modeling is based on studies of nonhuman islets or α cells. Since the cell arrangement and islet composition differ considerably in human islets, one must be cautious in extrapolating studies in rodent islets to glucagon secretion by human α cells. A representative glucagon secretory profile from islet perifusion and a schematic of presumptive α cell signaling pathways related to Ca^{2+} and cAMP with components that relate to the perifusion highlighted is shown in Fig. 6C and 6D.

While α cells express several of the same key components as β cells (GLUT1, GCK, and the K_{ATP} channel) (159,

360), α cells have different expression and localization of numerous ion channels, including voltage-dependent Na^+ channels and T-, L-, and P/Q-type Ca^{2+} channels, leading to a substantially different electrophysiologic profile (354, 356, 361). In the α cell, Ca^{2+} changes are modest, and oscillations are not as synchronous as they are in the β cell (354, 362). Thus, Ca^{2+} is likely playing a more complex and nuanced role in the control of glucagon secretion.

While the traditional role of the islet has centered on glucose control, recent work has highlighted that glucagon, in particular, has a fundamental role in regulating protein metabolism and amino acid homeostasis. Interrupting glucagon signaling in the liver leads to elevations in circulating amino acids, which in turn can induce α cell proliferation (363-368). In addition to regulating α cell mass, amino acids such as arginine, glutamine, and alanine have long been recognized as a strong stimulatory signal for glucagon secretion, which physiologically protects against insulin-induced hypoglycemia after a protein-rich meal (369-371). The cellular mechanisms behind amino acid-induced glucagon secretion are poorly defined but likely involve a combination of metabolic, electrical, and receptor-mediated processes depending on the individual amino acid (302, 372-375). The fact that amino acids can stimulate glucagon secretion independently of glucose has suggested the possibility that they play a primary role in glucagon secretion (305, 327, 376).

Lipids may also play a role in regulating glucagon secretion, though the precise effects and mechanisms in human islets have not been well studied (372). In humans, lipid ingestion or intravenous injection has varied effects on glucagon secretion (377, 378). Fatty acid stimulation of glucagon secretion is concentration dependent and varies with chain length. It is thought to be mediated by the G_q-coupled FFAR1 signaling through Ca^{2+} (379, 380).

Neurohormonal control

Circulating hormones can also modulate α cell function. Most notably, epinephrine, a strong stimulus for glucagon secretion as part of the counterregulatory response to hypoglycemia, can signal through multiple receptors. In the α cell it is thought to primarily signal through the β_2-adrenergic receptor, a G_s-coupled GPCR, and the α_1-adrenergic receptor, a G_q-coupled GPCR (381). Activation of both receptors may explain why epinephrine is such a potent stimulus, increasing both cAMP and Ca^{2+} within the α cell (382-384).

Paracrine signaling likely plays a fundamental role in the control of α cell secretion of glucagon (277, 279, 354, 385). Most notably, isolated α cells do not respond appropriately to stimuli (glucose in particular), which suggests that signals and interactions within the islet microenvironment are necessary for appropriate α cell function (386, 387). β cells are thought to be a regulator of glucagon secretion, with insulin being the prime mediator (388) as α cells express the insulin receptor. Other β cell–derived molecules include serotonin, which can act on G_i-coupled 5-HT_{1F} GPCRs on α cells to lower cAMP and inhibit glucagon secretion (347), and ATP, which can signal through G_q-coupled $P2Y_1$ receptors on α cells to increase intracellular Ca^{2+}. β cell–derived ATP may explain elevations in intracellular Ca^{2+} in α cells at high glucose despite reduced glucagon secretion (389, 390), thus providing a signal to balance the other inhibitory signals from β cells.

Somatostatin secretion from δ cells provides important local inhibition to the α cell (275). Like β cells, human α cells primarily express the SSTR2 receptor, a G_i-coupled GPCR shown to reduce cAMP in the α cell and robustly inhibit glucagon secretion (335, 391, 392). The unique distribution of δ cells has also led to numerous models whereby other signals ultimately affect glucagon secretion through δ cells. For example, acetylcholine, which is secreted by human α cells, can stimulate δ cells and thus provide indirect negative feedback (273, 278).

Finally, autocrine signaling by α cells may help regulate glucagon secretion. Glutamate, an abundant amino acid but also a major excitatory neurotransmitter, is packaged in α cell granules and co-secreted with glucagon (393). Human α cells express ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors capable of responding to glutamate by allowing Ca^{2+} and Na^+ entry into the cell; thus, signaling through these receptors both depolarizes the cell and increases intracellular Ca^{2+} to act as a positive autocrine signal (394). In addition, it has been suggested that α cells express G_s-coupled glucagon receptors that would signal by increasing cAMP as well (395). Thus, there are numerous potential pathways for positive autocrine signaling in the α cell, which may explain how relatively minor changes in other stimuli (such as glucose) can so effectively promote glucagon secretion (394).

Looking Forward: Topics to Explore Relating to Human Islet Physiology and Function

- What is the full array of secreted factors from endocrine and nonendocrine cells within the human islet?
- What is the role of electrical coupling and stimuli between human islet cells?
- How do peptide products from one islet cell type (eg, insulin, glucagon, GLP-1) influence hormone secretion by other islet cell types?

- How does intra-islet communication by nonpeptide molecules (metabolites, GABA, Zn^{2+}, etc) influence islet cell hormone secretion?
- What is the role of intra-islet somatostatin in human islet physiology and pathophysiology?

The Dynamic Islet in Physiology and Pathophysiology

While we have presented a classic "normal" human pancreatic islet in the preceding section, the islet is a dynamic mini-organ with both physiologic and pathophysiologic adaptations in islet cell composition, structure, and function over time (Fig. 7A). This section highlights some of these changes by providing a broad overview of human islet development, metabolism-driven changes during pregnancy, and cellular progressions that occur with aging. We also present emerging evidence for alterations during obesity and/or insulin resistance and review disease pathologies from well-characterized forms of diabetes (Fig. 7B and Table 6). Though these topics are narrated as dynamic processes, we emphasize that the current

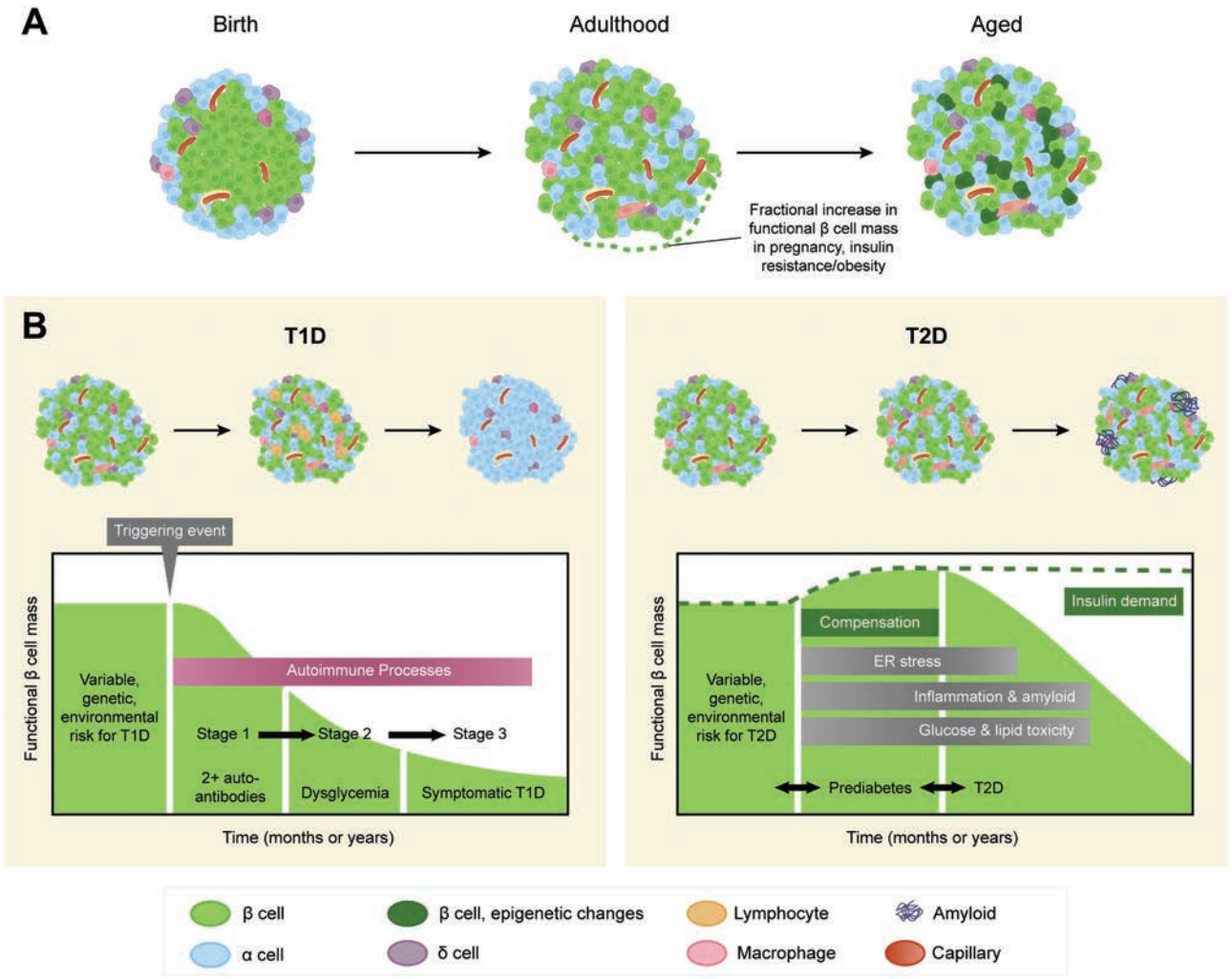


Figure 7. Age- and disease-related changes to islet structure and function. A, Schematic showing alterations to islet architecture and composition from birth to aging, based on cross-sectional histological evidence. At birth, islets contain a higher proportion of δ cells and lower proportion of β cells compared to adulthood. Additionally, β cells are located primarily in the islet core, whereas α and δ cells are primarily in the islet periphery. By childhood, endocrine cells are intermingled and β cells outnumber α and δ cells. In response to certain metabolic stressors such as insulin resistance/obesity or pregnancy, some studies have indicated slightly increased β cell mass. Aging is marked by epigenetic and molecular changes but maintenance of endocrine mass. B, Schematics showing development of type 1 (left) and type 2 (right) diabetes. In the type 1 diabetes (T1D) model, a yet-to-be-defined "triggering event" or multiple events are thought to initiate an autoimmune process, development of islet autoantibodies, and progressive loss of β cell mass. While this schematic depicts an islet containing only α and δ cells, the degree of β cell loss varies in individuals and with disease progression, and some β cells can still be detected in the pancreas of individuals with T1D. In the type 2 diabetes (T2D) model, progressively insufficient insulin secretion to meet (potentially elevated) insulin demand may be characterized by glucose and lipid toxicity and/or inflammation. In some cases, islet capillaries increase in size, macrophages infiltrate the islet, and/or amyloid deposits disrupt islet architecture. While curves showing changes in β cell mass are smooth, it is likely that the loss of functional β cell mass may stop and restart.

Table 6. Structural and functional changes to pancreatic islets in disease

Diabetes type	Islet/Pancreas structure	Islet function	Underlying genetics	Key references
T1D	• Drastic loss of β cell mass • Disordered islet cell organization • Immune cell infiltration • Abnormal extracellular matrix • Smaller pancreas with reduced acinar cell number	• Significant loss of insulin secretion • Possible β cell dysfunction during disease development • Evidence of nearly normal insulin secretion by residual β cells • Evidence of α cell dysfunction; impaired response to hypoglycemia	• Polygenic; some known heritability • Very strong HLA loci association • Other SNVs identified by GWAS are largely related to immune system	(113, 114, 124, 239, 243, 396, 397) Reviews: (398-401)
T2D	• Islets appear relatively normal early in disease • β cell mass variable depending on disease duration • Thickened islet capillaries and increased vessel density • Amyloid deposits in many, but not all, donors • Macrophage infiltration	• Reduced insulin secretion, particularly relative to demand (insulin resistance) • Evidence of α cell dysfunction; failure of glucagon suppression with meal	• Polygenic; some known heritability • SNVs identified by GWAS are largely related to islet cells • Many SNVs related to noncoding enhancer regions	(67, 211, 402-413) Reviews: (234, 400, 414-418)
GDM	• Largely unknown • Potential defect in compensatory β cell expansion	• Insufficient insulin secretion	• Polygenic; large overlap with T2D • Majority of loci related to β cell function • GDM-specific: *HKDC1*, *BACE2*	(419, 420) Reviews: (421-423)
MODY	• Variable depending on exact mutation; see text[a] • Case reports have described phenotypes including decreased β cell mass, impaired pancreatic morphogenesis, and pancreatic hypoplasia	• Variable depending on exact mutation; see text[a] • Case reports have described progressive β cell dysfunction due to insulin secretory defects, and/or glucose-sensing defects	• Monogenic • Mutations to *HNF4α*, *GCK*, *HNF1α*, *PDX1*, *HNF1β*, and *NEUROD1* constitute MODY 1-6, respectively • Other mutations identified, including those in *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, and *KCNJ11*	(424-434) Reviews: (435-437)
Neonatal diabetes	• Variable depending on exact mutation; see text[b] • Case reports have described phenotypes including pancreatic agenesis, pancreatic hypoplasia, and $CHGA^+$, $hormone^-$ cells	• Variable depending on exact mutation; see text[b]	• Monogenic; most commonly caused by mutations to *KCNJ11* or *ABCC8* • Other genes implicated include *FOXP3*, *GATA4*, *GATA6*, *GCK*, *HNF1β*, *INS*, *NEUROG3*, *PAX6*, *PDX1*, *PTF1A*, and *RFX6*	(267, 268, 438-444) Reviews: (445-448)

Table 6. Continued

Diabetes type	Islet/Pancreas structure	Islet function	Underlying genetics	Key references
PHHI	• Diffuse: disorganized islets; abnormal β cells in all portions of the pancreas; evidence of β and δ cell transcriptional abnormalities • Focal: abnormal β cell lesions; increased β cell proliferation	• Insulin hypersecretion resulting in severe hypoglycemia, caused by absent or dysfunctional K_{ATP} channel • Tumor development due to imbalance between imprinted genes mapped to 11p15 (focal only)	• Diffuse: mutation in *ABCC8* or *KCNJ11* • Focal: paternally inherited *ABCC8* or *KCNJ11* mutation ***and*** region-specific loss of maternal 11p15 alleles	(449-455) Reviews: (451, 456-459)
CFRD	• Increased immune cell infiltration of exocrine pancreas • Extensive fibrosis and fat infiltration (exocrine and peri-islet area) • Islet loss/dysmorphia	• In vivo insulin insufficiency likely due to islet loss • Remaining islets have similar function to normal islets in vitro	• Monogenic • Mutations to *CFTR* gene disrupt protein synthesis (class I/VI), processing (class II), or function (class III/IV)	(460-462) Reviews: (463-466)
Posttransplantation diabetes	• Largely unknown • Possible β cell morphologic changes • Impaired insulin granule formation	• Impaired insulin secretion, particularly related to certain immunosuppressive agents	• Polygenic; large overlap with T2D	(467-473) Reviews: (474-476)

Abbreviations: CFRD, cystic fibrosis-related diabetes; GDM, gestational diabetes; GWAS, genome-wide association study; HLA, human leukocyte antigen; MODY, mature-onset diabetes of the young (monogenic diabetes); PHHI, persistent hyperinsulinemic hypoglycemia of infancy; SNV, single-nucleotide variation (formerly single-nucleotide polymorphism [SNP]); T1D, type 1 diabetes; T2D, type 2 diabetes.
For further discussion of MODY, see [a]"Endocrine cell identity," paragraph 1, and "Other forms of diabetes," paragraph 2; for commentary on neonatal diabetes, see [b]"Other forms of diabetes," paragraph 3 and "Pancreas and islet development," paragraph 5.

understanding of age- and disease-driven shifts stem largely from cross-sectional studies. Despite advances in clinical imaging modalities and attempts to target human β cells in vivo, reliable and noninvasive imaging of functional β cell mass remains elusive (477, 478). Until such techniques reach fruition, a combination of biomarkers and postmortem tissue analyses remain the driving forces to understand how the pancreatic islet changes across the human lifespan and with disease. Thus, the following summary of events should be treated as evidence-based inference rather than defined fact.

Pancreas and Islet Development

The molecular mechanisms of pancreatic differentiation—including derivation from primordial gut, dorsal and ventral bud formation, epithelial migration, and branching morphogenesis—are thought to be largely conserved between the mouse and human, though human endocrine cells develop over a more sustained period as opposed to distinct "primary" and "secondary" transitions in the mouse (248, 249, 479, 480). Parallels between mouse and human pancreatic development (89, 255, 481), as well as in-depth discussion of developmental stages (479, 480), have been nicely reviewed elsewhere; this commentary will summarize key events in human pancreas and islet development.

Human endocrine cells arise from multipotent pancreatic progenitor cells (MPCs), which have been identified in fixed tissue as well as manipulated ex vivo (482, 483). Recent transcriptomic and proteomic characterization has begun to provide insight into the markers and mechanisms of differentiation, including the identification of SOX9 and PTF1A expression in epithelial cell "tip" progenitors that promote expansion through *MYC* and *GATA6* (484). Endothelial cells are thought to support the progenitor niche through secretion of EGF, with FGF10 and R-spondin1 also stimulating progenitor proliferation ex vivo (485, 486). Glycoprotein 2, a cell surface marker of MPCs both in human tissue and stem cell–derived cultures (487-489), also marks the multipotent population (489). MPCs are thought to commit to endocrine/ductal or acinar lineages through upregulation of NKX6-1 and PTF1A, respectively, with mechanistic studies relying heavily on stem cell models to confirm these cell-fate decisions (94, 490, 491).

Beginning at approximately 8 weeks of gestation, endocrine cells start to differentiate within the developing epithelial tubes (126, 492, 493). Numerous studies suggest that β cells are the first to appear, and these insulin-expressing

cells remain the most abundant of all endocrine cell types throughout the first trimester (494, 495). Differentiating β cells are followed by α and δ cells, at around 8 to 9 weeks of gestation, and by γ and ε cells shortly thereafter (494-497). Bihormonal cells have been reported to varying degrees (493-495, 498, 499), but ultrastructural analysis indicates that many differentiating endocrine cells contain a mix of granules resembling those in mature α and β cells (126). Of interest is the observation that INS^+ GCG^+ cells expressed ARX but not PDX1, NKX6-1, or MAFA, suggesting they were likely to become α and not β cells (494). As detected by immunohistochemistry and/or quantitative reverse transcriptase–polymerase chain reaction, endocrine cells appear to upregulate NGN3, ISL1, NEUROD1, NKX2-2, and PAX6 between 8 and 12 weeks, with PDX1 and NKX6-1 being specific to β cells (249). Early endocrine cells are closely associated with endothelial cells (496, 500) and $CD68^+$ macrophages are present; expression of chemokines by epithelial cells and chemokine receptors by both epithelial and mesenchymal cells suggests immune cell recruitment may be involved in early endocrine cell differentiation (492, 501, 502).

Islet-like clusters form and delaminate from the epithelium starting around 12 weeks of gestation, composed primarily of α and β cells (497) and containing ECM networks made up of collagen I, collagen IV, fibronectin, and laminin (126). Blood vessels with a prominent smooth muscle cell coverage, along with lymphatic vessels, are discernable at the beginning of the second trimester in pancreatic regions outside islets (493, 496, 500). Blind capillary spouts and multiple portal connections provide evidence of dynamic angiogenesis and remodeling, and by the end of the second trimester, vascular architecture is almost completely developed (125, 498, 503). Nerve terminals appear about the same time as vasculature, just after the growth factor neuron-specific enolase is detected in endocrine cells (504). Close association of neurons and islets is observed midgestation, at approximately 24 weeks, and later (504, 505). Nerve fibers terminating in the fetal pancreas are most dense in the head region, where the area, perimeter, and width of nerves increases from 14 to 22 weeks (506) and then reduces. Interestingly, these dynamic changes correspond to remodeling of the intrahepatic biliary system (506).

The internal and systemic signals driving human endocrine cell development have been extrapolated from cross-sectional immunohistochemical studies, from modeling cell differentiation using induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs), and from documenting phenotypes of neonatal diabetes caused by mutations in key genes implicated in pancreatic and endocrine cell development. For example, certain mutations in *NEUROG3*, which is expressed late in the first trimester but decreased by the start of the third trimester (495, 507), cause neonatal diabetes by preventing NEUROG3 from binding to the *NEUROD1* promoter (267, 445). However, other patients with a functionally "null" variant did not develop diabetes until later in childhood, suggesting compensatory pathways (508). Downstream of *NEUROG3*, *RFX6* has also been implicated in multiple cases of neonatal and or delayed-onset neonatal diabetes, presumably resulting from impaired endocrine cell differentiation (442, 443).

Beyond *NEUROG3* and *RFX6*, which are required generally for appropriate endocrine cell differentiation (94), *NKX6-1* and *MAFA* are specifically important for β cell lineage allocation and maturation (509). Transcription factor *ARX* is essential to human α cell development (94), with *IRX2* and *MAFB* also known as signatures of this cell population. The location and growth trajectory of PP-expressing cells in the fetal pancreas suggest a lineage relationship between α and γ cells (499), though transcriptional profiles of γ and ε cells are limited because of their scarcity in human islets. Transcription factors *PAX4*, *PDX1*, and *HHEX* appear central to the δ cell lineage (510, 511). Moreover, external stimuli, including endocannabinoids (512), semaphorin/neuropilin signaling (513), and retinoic acid (514), have all been recently implicated in human endocrine cell maturation and/or islet formation.

Until the last decade, it was largely unknown whether endocrine cells in the developing human pancreas arose from proliferation of terminally differentiated cells or from multipotent pancreatic progenitors that were renewed throughout the fetal period. In pioneering studies, Scharfmann and colleagues (483) transduced human fetal pancreatic explants at low multiplicity of infection with lentivirus expressing green fluorescent protein under the rat insulin promoter, effectively labeling only a subset of progenitors, and then allowed cells to mature following transplant into immunodeficient mice. After 4 weeks they observed a relatively low proportion of green fluorescent protein–labeled β cells within a subset of islets, supportive of considerable de novo endocrine cell formation. Additionally, proliferating endocrine cells have been noted in a number of other studies (492, 493, 497, 498), thus indicating that before birth, islet endocrine mass in humans, unlike in mice, is established through processes of both endocrine cell differentiation and proliferation. Variable rates of apoptosis have been reported throughout fetal development but are generally low (450, 493). Populations of α, β, and δ cells appear to change dynamically during fetal development and at time of birth; islets at this stage contain more δ cells compared to late childhood and adulthood (494, 497, 505), suggesting that α and β cell populations grow at a higher rate than δ cells postnatally, which would represent another divergence from mouse pancreas development.

While investigation of the fetal human pancreas has uncovered key morphogenic and cellular processes, the period of islet development after birth and the first decade of life remains far less characterized. A prominent report by Gregg and colleagues in 2012 (505) documented a burst in β cell proliferation within the first 1 to 2 years of life, with evidence of elevated endocrine cell apoptosis during the late fetal and early postnatal period that swiftly declined by age 6 months (450). The β:α cell ratio doubled in the years after birth, with a 5- to 7-fold increase in the ratio of β:δ cells (200, 505, 515). In a recent study, Cogger et al reported that putative PTF1A$^+$ NKX6-1$^+$ GP2$^+$ multipotent progenitors, previously identified in tips of epithelial tubes during late gestation (488), persist at birth, raising a possibility that islet neogenesis may continue postnatally and contribute to endocrine mass expansion. Interestingly, many human islets at birth resemble those of rodents—a β cell core surrounded by mantle of α and δ cells—which are distinct from the intermingled arrangement of α, β, and δ cells in adult islets (19, 193, 505). How and why this shift occurs is largely unknown, but such dynamic changes likely affect the risk for and/or development of T1D and T2D, and more studies are needed to define these critical processes in the young human pancreas.

Looking Forward: Topics to Explore Related to Human Pancreas and Islet Development

- How does the in utero environment (material diet, health, etc) affect human islet development and subsequent human islet function and mass?
- What critical events in islet development (in utero, during the neonatal period, and first decade of life) influence islet cell differentiation, gene expression, proliferation, and composition?
- What are the genetic, environmental, and nutritional determinants of β, α, and islet cell mass—can these be influenced?

Pregnancy

To meet metabolic demands of the developing fetus, maternal insulin sensitivity progressively declines and circulating fatty acids and glucose levels increase. Studies in rodents suggest that pancreatic β cells and islets compensate through increased insulin production and hypertrophy/hyperplasia; however, our knowledge of specific structural and functional changes in the human islet during pregnancy or during gestational diabetes is quite limited. Almost all the information about islet changes during pregnancy come from animal models (516, 517), with only a few histologic autopsy studies of the pancreas from individuals who were pregnant or recently pregnant at the time of death (518, 519). Based on these limited analyses, it is estimated that fractional pancreatic β cell area increases 1.4- to 1.7-fold during human pregnancy, with no apparent change to cell size and no detection of increased β cell replication or apoptosis rate (though limitations of cross-sectional studies apply) (518, 519). If correct, this is a major difference in how the islet changes in nonhuman models during pregnancy.

Pregnancy-related hormonal changes have guided investigation of signaling pathways that might be involved in adaptions of the β cell and islet to increased metabolic demands. Lactogens in particular have received attention for their ability to stimulate β cell proliferation and confer protection from metabolic stress in vitro in rodent systems (520-523). For example, prolactin and its receptor are central to rodent islet response during pregnancy; however, adult human β cells neither express the prolactin receptor nor show a proliferative response to prolactin receptor signaling (524). Serotonin, which is elevated in islets during pregnancy (348), has also been implicated in enhancement of glucose-stimulated insulin secretion in nonhuman systems, but its role in human islet physiology during pregnancy has not yet been defined (525). A number of other hormones—corticotropins (526-528), estrogens (529), prostaglandins (530-532)—have known effects on human β cells and may contribute to adaptions. Recent work arising from genome-wide association study (GWAS) data has shed light on possible roles for chromatin remodeling factor HMG20A and transcription factors PAX4 and PAX8 in the adaptation of β cells to pregnancy (422, 533). More details are found in recent reviews (422, 534).

Aging

How human islets change during the aging process has not been extensively studied, despite age being a strong risk factor for T2D (535). There are reports of lower rates of proliferation with advanced age, which would fit with the age dependence of proliferation in human β cells (536-539). However, given the extremely low rates of proliferation in the adult human β cell population to begin with, it is difficult to know the significance of this finding. As postmitotic cells, individual islet cells have little turnover and estimations using accumulated lipofuscin, a marker of aging, indicate that more than 95% of all α or β cells are formed prior to age 20, underscoring the importance of developmental processes in establishing an individual's β cell mass (540, 541). Several studies have found that despite higher rates of diabetes in the population, β and α cell mass are both largely maintained in nondiabetic individuals with advanced age (542-544). These results suggest that the capacity for β cell functional adaptation to stress and/or metabolic demand may be reduced instead

(545). While insulin sensitivity tends to decrease with age, in vivo insulin secretion has been found to either decrease with age or fail to show the expected compensatory increase given the change in insulin sensitivity (546-551). In isolated mouse islets, insulin secretion dynamics declined with age in a process that may be mediated by disrupted gap junction coupling of β cells, leading to reduced coordination of intracellular Ca^{2+} dynamics—little is known about these changes in human islets (320, 552, 553). Further, mitochondrial function has been shown to decline with age and may also be a source of compromised function (554, 555).

Numerous cellular processes have been postulated to explain these age-related functional changes. In contrast to mice, human islet cells appear to accumulate intracellular lipid in an age-dependent manner, and the long-term effects of intracellular lipids in lipofuscin bodies or lipid droplets in islet cells is not known (556-558). Recently, cellular senescence, a process of cell cycle arrest and acquisition of a senescence-associated secretory phenotype characterized by secretion of proinflammatory cytokines and chemokines, has been highlighted in aging (559). However, there are conflicting reports on whether this process in β cells enhances or disrupts insulin secretion and thus the specific context likely plays an important role (539, 560-562). In addition to the β cell, other cells within the islet are also subject to an aging-related decline. For example, aged endothelial cells are more sensitive to oxidative stress and can be a source of proinflammatory signals (563-565). Interestingly, aged murine pancreatic islets transplanted into young mice showed improved function compared to aged mice, highlighting the role of nonislet cells in the microenvironment, specifically capillary vessels, in islet function and dysfunction with age (566). Again, our knowledge of these processes in human islets is quite limited.

In addition to disrupted signaling pathways and systemic factors, intrinsic changes in the epigenetic landscape of individual cells have emerged as a major factor in aging (567). In islets, age-associated epigenetic alterations correlated with changes in insulin secretion (568, 569). Further, single-cell analyses of islets from donors with advanced age showed evidence of transcriptional noise and fate drift thought to result from epigenetic alterations as well as somatic mutation patterns (570, 571). In sum, these results highlight the need to better understand both the intrinsic and environment-related changes that occur in islets with aging.

Obesity

Metabolic impairments such as obesity are associated with, but not required for, the development of T2D. A majority of individuals with T2D meet the criteria for being overweight (body mass index [BMI] ≥ 25) or obese (BMI ≥ 30) (572, 573), and obesity is highly correlated with insulin resistance and hyperinsulinemia. Accordingly, insulin secretion both in vivo and in vitro positively correlates with increases in BMI (145, 574, 575). The prevailing view is that obesity and insulin resistance are factors that β cells respond to by elevating insulin secretion; however, an alternative proposal has emerged in which insulin hypersecretion may play a causal role in obesity, insulin resistance, and eventually T2D (576-579).

In addition to elevated insulin secretion, some autopsy studies have suggested that nondiabetic obese donors may have greater β and α cell mass, perhaps reflecting an increased capacity for insulin secretion while maintaining a balanced ratio between β and α cells (410, 580-582). Importantly, there is considerable overlap of β mass in donors across a range of BMI and other studies have not seen an effect of obesity on increased β cell mass (583). To explain this rough association of β mass and obesity, a number of mechanisms of obesity-induced β cell proliferation, including as a response to liver-derived factors such as SerpinB1, have been proposed (584-586). However, in contrast to mouse β cells, human β cells from islets transplanted into mice on a high-fat diet did not show an increase in β cell proliferation (558, 587). Consistent with this, the same studies that found greater β cell mass in obese donors did not find a greater number of $Ki67^+$ β cells, highlighting the difficulty of studying β cell mass dynamics in humans and suggesting that the proliferative capacity of adult β cells is likely quite low.

In addition to effects mediated through insulin resistance, obesity is associated with increased inflammation (588, 589). Obesity-associated islet inflammation is thought to be characterized by increased islet macrophage accumulation as well as a change in polarity toward a more proinflammatory phenotype (590-592). These altered macrophages appear to have numerous effects on the islet microenvironment, including secreting factors that may promote β cell expansion (593, 594). At the same time, the shift from anti-inflammatory to proinflammatory cytokines appears to contribute to β cell dysfunction and may establish an intra-islet milieu that contributes to increased susceptibility to T2D in individuals with obesity (595, 596).

Looking Forward: Topics to Explore Relating to the Human Islet Response to Pregnancy, Aging, and Obesity

- What are the adaptations in the human islet during and after pregnancy (mass, function, islet cell composition, etc)? How does gestational diabetes affect subsequent islet mass and function?

- What human islet adaptations allow some obese individuals to maintain glucose homeostasis while others progress to diabetes?
- What are the epigenetic changes (intrinsic or environmentally induced) in human islets with age or disease and how do they affect islet cell function, survival, and adaptation?

Disease

The classification of diabetes is still quite rudimentary, with the categories of diabetes based on clinical criteria rather than molecular pathogenesis. While most forms of human diabetes are associated with impaired islet cell function and/or reduced β cell mass, the molecular events and mechanisms leading to dysfunction or reduced mass in different forms of human diabetes are either incompletely characterized or largely unknown (597, 598). These knowledge gaps result primarily from the limitations and difficulties in studying the human islet and pancreas, which are discussed in the introduction of this review. Importantly, many new and emerging technologies described in Table 3 have not yet been applied to the study of the pancreas from humans with diabetes, mostly because the donor organs are not procured in a timely fashion or not processed in a way that allows detailed analysis. Fortunately, this is beginning to change with focused efforts in Europe and the United States to apply these new technologies to the human pancreas from donors with diabetes (as mentioned earlier, the Network for Pancreatic Organ donors with Diabetes, the Human Pancreas Analysis Program, the Innovative Medicines Initiative for Diabetes, etc). As new findings are being integrated with earlier, autopsy-based studies of the human pancreas in diabetes, the structural, functional, and cellular changes in the human islet are beginning to emerge. The current status of knowledge about the human islet and pancreas in common forms of diabetes is summarized as follows and in Table 6.

Type 1 Diabetes

T1D is characterized by a dysregulated autoimmune response of both the adaptive and innate immune system, ultimately resulting in the destruction of β cells (399, 599). Recent consensus divides the natural progression of T1D into 3 stages (Fig. 7B). Stage 1 is characterized by the presence of 2 or more islet autoantibodies and is thought to mark the initiation of β cell loss despite the maintenance of normoglycemia (600, 601). The autoimmune process is thought to be initiated or potentiated by a triggering event, although what this may be is not known. Stage 2 is characterized by dysglycemia and dysfunctional insulin secretion in response to a glucose challenge, whereas stage 3 is characterized by symptom onset and is thought to occur after the loss of approximately 60% to 90% of an individual's β cell mass, though exact quantification is not currently feasible (602). Despite this general model, there is poorly understood T1D heterogeneity in terms of age of onset, rate of disease progression, and residual C-peptide production (399, 603). For example, one report estimated that as many as 40% of T1D patients developed the disease after age 30 years (603), while T1D has also been reported to occur within the first 6 months of life (604). Borrowing a paradigm from other diseases with clinical heterogeneity, the emerging concept is that there are "endotypes" of T1D based on incompletely defined genetics and pathologic processes (605, 606).

Islet-immune interactions are crucial in T1D. Modest insulitis, or lymphocytic infiltration of the islet, is a hallmark pathologic feature of T1D—though there is significant variability in the cellular composition and frequency of insulitis among donors (607, 608). Insulitis is often characterized by tight focal aggregation of immune cells at one islet pole and the immune cells are primarily CD8+ T cells, though β cells, CD4+ T cells, and macrophages may also be present (399, 609-611). Islet β cells in T1D show elevated expression of human leukocyte antigen class I and class II components, potentially facilitating autoimmune surveillance and destruction (612, 613). Furthermore, a majority of identified genetic loci associated with T1D are linked to immune-related genes, with human leukocyte antigen loci accounting for more than 50% of the risk (399, 601). Understanding the role that β cells play in the autoimmune process is of great interest, with the growing sense that β cells or the β cell response contributes to β cell demise (614). Recent multiplexed imaging studies have highlighted that prior to destruction, β cells lose markers of cell identity and show altered protein expression, though it is unclear if these changes are indicative of adaptations to avoid immune detection or pathologic changes that invite destructive, autoreactive T cells (113, 114). Interestingly, β cells that remain in T1D appear to have nearly normal insulin secretion profiles, highlighting that T1D defects are primarily related to changes in β cell mass rather than function (396).

While T1D pathophysiology is primarily focused on β cells, there is evidence for the involvement of other cell types in the pancreas. Individuals with T1D have an impaired counterregulatory response that can lead to potentially dangerous hypoglycemia. This defect is multifactorial but appears to involve dysregulated glucagon secretion and compromised gene expression in α cells in T1D (396, 615-617); how the α cell responds to the immune and metabolic stresses of T1D, as well as to the loss of local paracrine

signaling from β cells and disrupted islet architecture, will be important to define going forward. Further, there is emerging evidence for the involvement of the entire pancreas in T1D pathogenesis, as individuals with T1D have significantly smaller pancreas size characterized by a loss of acinar cell number, highlighting an important, but understudied, interaction of islet pathology with exocrine tissue (618-621).

Type 2 Diabetes

T2D is a very heterogeneous disorder from a clinical standpoint, with likely multiple molecular pathways and time courses to reach hyperglycemia. T2D is characterized by islet dysfunction, defined by insufficient insulin secretion from β cells and inappropriate glucagon secretion, often on a background of peripheral insulin resistance that arises in states such as obesity or advancing age (417). Insulin resistance in T2D tends to remain relatively stable throughout disease while β cell functional mass declines, highlighting both initial and progressive β cell failure as a key determinant of T2D pathogenesis (Fig. 7B) (622). This decline in insulin production mirrors the clinical disease course for which escalating treatment paradigms are needed to promote glucose homeostasis (623). Rather than insufficient insulin secretion, an alternate hypothesis for the sequence of events leading to T2D is that insulin hypersecretion and subsequent hyperinsulinemia is the initial defect, with the hyperinsulinemia leading to obesity and insulin resistance that eventually results in β cell failure (576).

While there are ongoing arguments about whether T2D is accompanied by reduced β cell mass or reduced β cell function, most favor a combination of the two. For example, cross-sectional postmortem studies suggest a mild reduction in β cell mass in T2D, but there is significant overlap in β cell mass among T2D and normal individuals. Thus, it remains unclear whether this mild mass reduction is the result of disease-associated β cell loss or merely a different baseline in β cell mass that gives rise to differential susceptibility to T2D (410, 581, 583, 624-626). The central role of the β cell is further highlighted in GWAS studies, where the majority of the loci identified are related to β cell biology (408, 409, 627-629). The identified GWAS variants, which collectively explain only a small proportion of the overall genetic risk attributed to T2D, lie largely in noncoding regions that may allow them to have broad effects on β cell processes and function, but makes specific study of their effects challenging (407, 630, 631). How most of the GWAS-defined loci contribute to T2D is still unclear, with many studies underway to examine the impact on islet function.

Mirroring the clinical heterogeneity in T2D, molecular studies suggest considerable variability and complexity in defects leading to inadequate insulin secretion. Indeed, there is increasing evidence that points to a complicated interplay of stress pathways and impaired β cell function as a major driver of decreases in β cell functional mass (67, 180, 412). Components such as glucotoxicity and lipotoxicity and chronic inflammation are proposed to cause activation of stress pathways in the islet, including endoplasmic reticulum (ER) stress, oxidative stress, cytokine stress, and hypoxic stress (558, 632-635). However, many of these processes have been studied only in human islets manipulated in vitro and thus, the actual molecular events remain uncertain. In addition, a subset of T2D islets shows amyloid, an aggregation of fibrillary islet amyloid polypeptide hormone that is normally co-secreted from β cells (625, 636, 637). This striking pathologic hallmark has prompted significant investigation into the pathologic processing that underlies aggregation in T2D islets, as well as whether the intermediate oligomers formed during amyloid formation or the end deposits themselves cause further stress to the islet (404, 405, 638-641). These processes remain incompletely understood but are the topic of many ongoing studies.

While the β cell may initially be able to compensate for elevated stress, islet function eventually fails and results in processes that may include dysregulated secretion, autophagy, loss of cell identity, dedifferentiation, and/or apoptosis. Despite this general paradigm, it should be noted that there is likely great variability in the relative contribution, temporal sequence, and underlying etiologies of these components in different populations and individual patients, reflecting individual differences in genetics and environment (417, 642). For example, T2D in youth is associated with faster and more substantial β cell deterioration than T2D in adults, underscored by a different response to diabetes-directed therapies (643). This complex heterogeneity highlights the difficulty in making precise mechanistic determinations about islet dysfunction in T2D.

While the focus is primarily on β cells, islet dysfunction in T2D involves other cell types as well. Notably, dysregulated glucagon secretion from α cells, particularly apparent with the failure glucagon suppression after a meal, results in increased hepatic glucose output and can exacerbate insulin insufficiency (644, 645). More work is needed to identify whether this α cell dysfunction in T2D results from intrinsic α cell defects or from the loss of appropriate paracrine signals from β cells. Additionally, T2D is associated with disruptions to nonendocrine cells including macrophages, endothelial cells, and pericytes that aid in overall function (217, 221, 590, 646). In particular,

amyloid deposits in the T2D have been proposed to activate intra-islet macrophages and have also been shown to disrupt intra-islet vasculature (211, 647, 648).

Other Forms of Diabetes

Although T1D and T2D are most common, there are many other forms of diabetes, and this review mentions a few relevant to the human islet biology (discussed earlier; see Table 6). Gestational diabetes mellitus (GDM) occurs in individuals who cannot appropriately respond to the metabolic challenges and insulin resistance of pregnancy (420, 421, 423, 649). After resolution of pregnancy, most individuals with GDM return to normoglycemia, but they are at significant risk for the future development of T2D (650). As mentioned earlier, the molecular pathogenesis in GDM is not known. GDM may also be a clinical marker for some forms of monogenic diabetes (651, 652).

In addition to polygenic forms of diabetes, there are also numerous monogenic forms of diabetes—these have greatly advanced the understanding of human islet development and function by highlighting critical genes for β cell differentiation, maturation, and insulin secretion. MODY is classically defined as monogenic diabetes with (1) onset before age 25 years, (2) autosomal dominance inheritance, and (3) absence of autoimmunity (436). While mutations have been identified in more than 15 different genes, some familial forms of monogenic diabetes have no mutation yet defined. Identified subtypes generally are defined by mutations to genes related to transcriptional regulation (MODY1: *HNF4A*, MODY3: *HNF1A*, MODY4: *PDX1*, MODY5: *HNF1B*, MODY6: *NEUROD1*, MODY7: *KLF11*, MODY9: *PAX4*, MODY11: *BLK*), enzyme disorders (MODY2: *GCK*), protein misfolding (MODY8: *CEL*, MODY10: *INS*), ion channels (MODY12: *ABCC8*, MODY13: *KCNJ11*), and signal transduction (MODY14: *APPL1*), with MODY2 and MODY3 being the most common (653-655). MODY subtypes are unified by their cause of a β cell defect and disruption of insulin release but generally have their own unique clinical, functional, and structural characteristics that have provided important clues into the role of the underlying genes in human islet biology (95, 429, 436).

In contrast to MODY, neonatal diabetes describes a monogenic form of diabetes that presents within the first 6 months of life. Like MODY, these mutations can lead to diabetes in a variety of ways but are unified in having a substantial effect on islets and β cells (447, 448). Common causes of neonatal diabetes include activating mutations in K_{ATP} channel genes *KCNJ11* or *ABCC8*, which misregulate channel opening and prevent insulin secretion (438, 439, 446). In contrast, inactivating mutations in *KCNJ11* or *ABCC8* lead to inappropriate insulin secretion and hyperinsulinism (455, 458, 459). Mutations in the insulin gene or islet-enriched transcription factors can also cause neonatal diabetes (447, 448).

Pancreatogenic diabetes, meaning diabetes resulting from disease processes in the exocrine pancreas such as chronic pancreatitis or a mutation in carboxyl-ester lipase, highlight the connection between the endocrine and exocrine pancreas (656). Cystic fibrosis–related diabetes (CFRD) has become more frequent with the improved clinical outcomes in cystic fibrosis. CFRD usually requires insulin treatment, with reduced insulin secretion likely caused by islet loss, dysmorphia, and dysfunction that results from pronounced exocrine destruction and infiltration of immune cells, especially T cells (461, 466). This pathology is not a direct impact of *CFTR* mutations in β cells but rather is the result of *CFTR* mutations in the exocrine pancreas (461, 466, 656).

Posttransplantation diabetes, which also shares numerous risk factors with T2D, is a common but significant complication after organ and cell transplantation that threatens the health both of the graft and the transplant recipient (474, 475). Posttransplantation diabetes is likely multifactorial but stems in large part from β cell dysfunction induced by immunosuppressive agents (467, 472, 473).

Looking Forward: Topics to Explore Relating to Islet Alterations in Disease

- How do we classify an individual's diabetes based on genetics and the molecular and functional characteristics of islet cells and/or islet cell mass?
- What is the natural history of β cell loss or dysfunction in T1D and T2D?
- What are the key molecular processes that lead to islet dysfunction in T2D and do these differ depending on ethnicity and age of diabetes onset?
- What is the role of amyloid in islets in T2D?
- Is the loss of exocrine cells in T1D related to the loss of β cells?
- Are insulin and glucagon secretory defects in recent-onset T2D similar to those in long-standing T2D and are they reversible?

Looking Forward: Needed Experimental Approaches and Resources

- Ability to noninvasively and safely assess β mass in humans

- Reliable methods for targeting β cells in vivo to deliver new therapeutics
- More robust mechanisms to collect pancreatic tissue and islets from clinically phenotyped individuals across the spectrum of age, BMI, and ethnicity, and from individuals at risk for diabetes and individuals in different stages of human diabetes; study of these tissues and islets by multimodal investigation
- Approaches to generate mechanistic insights into how polygenic genetic loci (from GWAS, etc) influence diabetes (T1D and T2D) susceptibility and pathogenesis

Therapeutic Implications and Clinical Strategies

Given the pancreatic islet's central role in all forms of diabetes, it follows that many new or emerging therapeutic approaches focus on affecting islets—especially preserving, replacing, or enhancing β cell mass. Most emerging strategies have not yet been tested in humans with diabetes but can be divided into 2 broad categories: 1) β cell replacement and 2) maintenance, expansion, or modulation of functional β cell mass (Fig. 8). In T1D, strategies also include immunomodulation of the autoimmune response (399, 599).

β Cell Replacement

In T1D and in some individuals with T2D, it would be highly desirable to replace or supplement the inadequate β cell mass. Within the approach of β cell replacement or transplantation, this section describes 2 sources of insulin-producing cells, one of which is islet transplantation (which replaces more than just β cells) and one of which uses insulin-producing cells that are derived from other cells but are technically not β cells. Whole pancreas transplantation is sometimes an option when combined with renal transplantation (657).

β Cell Transplantation Using Human Islets

Islet allotransplantation in combination with immunosuppression has been the focus of intense efforts by many groups since the improved results of Shapiro and colleagues in 2000 (41). In this procedure, normal human islets isolated from cadaveric donor(s) are infused into the portal

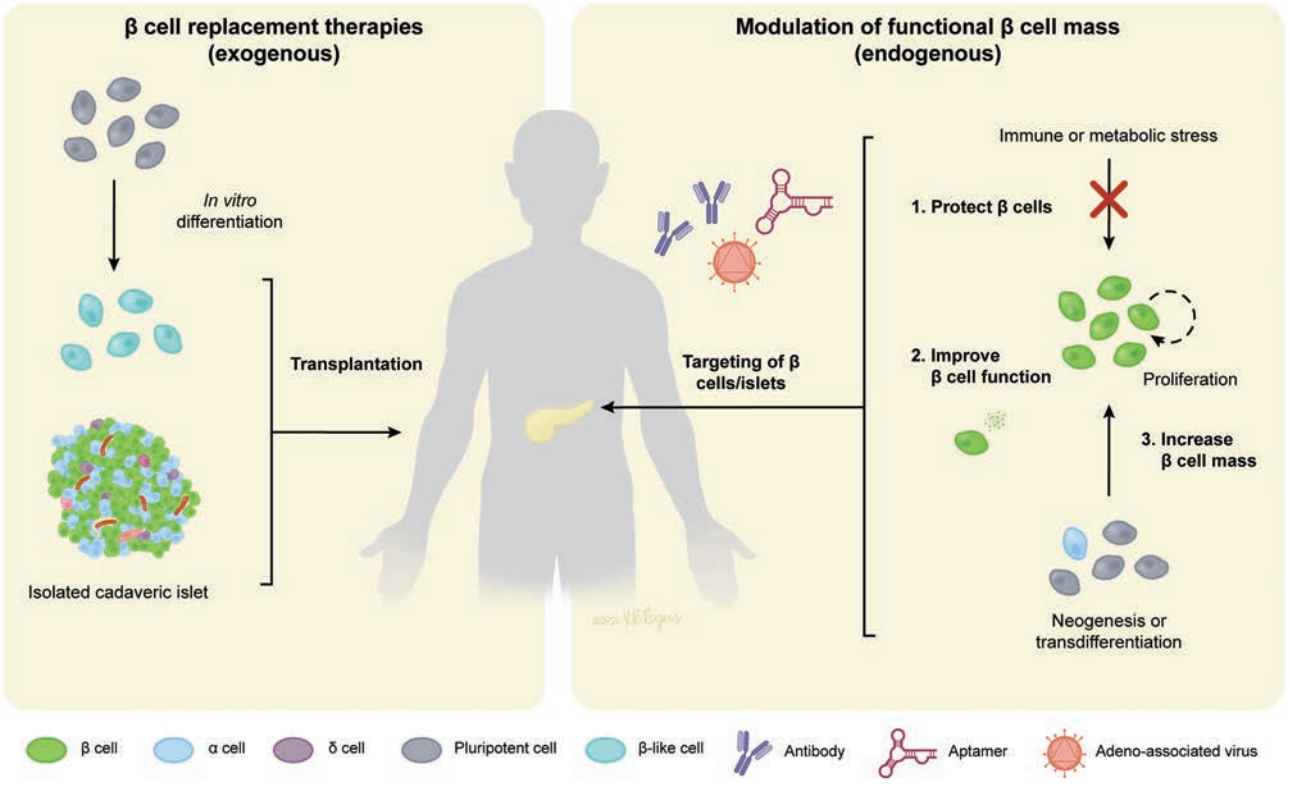


Figure 8. Clinical strategies to restore functional β cell mass. Exogenous β cell replacement approaches (left panel) include transplantation of cadaveric islet (human or xenograft) or of stem cell-derived β-like cells. Endogenous approaches (right panel) can be categorized into those that 1) protect β cells from immune or metabolic stress, 2) increase β cell mass through proliferation, neogenesis, or transdifferentiation, and 3) improve β cell function. Modulation of these strategies may require use of β cell or islet-targeting approaches such as antibodies, aptamers or adeno-associated viruses.

vein (percutaneous transhepatic portal vein delivery) with subsequent engraftment in distal liver vasculature (657-659). This approach, which benefits from the use of mature, fully functioning islets with relatively intact microenvironment and cell composition, has been effective in ameliorating life-threatening, severe hypoglycemia (49). Based on a phase 3 trial conducted by the National Institutes of Health–sponsored Clinical Islet Transplantation Consortium (44), several US centers are working to file a biologic license application with the Food and Drug Administration, which has recently issued guidance on the isolation and preparation of islets for future clinical transplantation for life-threatening, severe hypoglycemia (https://www.fda.gov/media/77497/download). Similar efforts in Europe are focused on islet transplantation (https://ecit.dri-sanraffaele.org/). Reduced hypoglycemia and improved quality of life after islet-after-kidney transplantation have been recently reported (660), but this therapeutic approach faces a number of significant challenges, including islet loss in the posttransplantation period, the need for lifelong immune modulation to prevent ongoing alloimmunity and autoimmunity, the need for islets from more than one donor pancreas in some transplant recipients, and β cell toxicity from common immunosuppressive agents (49, 467, 474, 658, 661, 662). A 20-year follow-up of islet transplant recipients at one center reported a mean duration of islet graft function of 4.4 years on immunosuppression (663). Even if such challenges are overcome, the very limited supply of human islets will not allow the widespread adoption of islet transplantation for T1D (657).

The clinical outcomes are improving for total pancreatectomy and islet autotransplantation, used to treat intractable pain related to severe recurrent acute or chronic pancreatitis, and this procedure is now being performed more frequently and earlier in the course of chronic pancreatitis (55). Whole pancreas transplantation is relatively uncommon and is mostly performed in the setting of renal transplantation, with either simultaneous pancreas-kidney transplantation or pancreas-after-kidney transplantation (657, 664).

β Cell Transplantation Using Other Sources of Insulin-Producing Cells

To develop a new source of β cells for transplantation, intense and ongoing efforts are directed toward the creation of human β-like cells using human ESCs or human iPSCs and toward identifying xenograft-based approaches (porcine) (657). Using knowledge from developmental islet biology, investigators have developed protocols involving sequential stimulation and the inhibition of specific developmental pathways with growth factors and small molecules to generate insulin-producing cells that are glucose responsive and can reverse diabetes in mice (86-88, 665). In contrast to most islet transplant procedures, iPSCs could use a patient's own cells and remove alloimmunity concerns (666), though there are also efforts to generate islet-like cells capable of evading immune detection (667). Directed differentiation is a rapidly evolving area of research with many recent protocol modifications, such as endocrine cell clustering (668), circadian entrainment (91), estrogen-related receptor γ expression (669), and enhanced transforming growth factor β signaling (90). Current efforts are focused on speeding up and refining the maturation process, improving dynamic insulin secretion, generating monohormonal cells. While this is an exciting area of research, there are important questions, including the safety profile, before transplantation of insulin-producing cells can move into the clinical area. For example, safety concerns about undifferentiated cells becoming transformed after transplantation remain, especially if cells were allografts and immunosuppression was needed. Additional questions include 1) how many insulin-producing cells are needed for diabetes reversal as insulin production in these cells is less than that of native human islets; 2) how long these cells will survive and function after transplantation, and 3) if transplantation of insulin-producing cells alone will be sufficient to restore glucose homeostasis or whether a more complete islet microenvironment involving glucagon-producing cells (92) or other components of the native islet will be required.

Maintenance, Expansion, or Modulation of Functional β Cell Mass

In many forms of diabetes, β cells remain but are not capable of meeting insulin demands. Here, therapeutic approaches seek to restore or bolster β cell function and maintain or expand β cell mass. In the cases of expanding β cell mass, there is also a need to ensure that the newly generated β cells are fully functional and ultimately; there may be an opportunity to combine approaches that stimulate β cell proliferation with those that bolster β cell function.

Improving or Preserving β Cell Function

While attractive, this has been a difficult therapeutic path since our current understanding of the reason(s) for impaired β cell function, and thus the target of intervention, is quite limited. As discussed earlier for T2D, multiple abnormalities have been postulated, but it is not known if one abnormality is primary or if there are multiple pathways to β cell dysfunction. The progressive nature of T2D indicates that most current antihyperglycemic medications

such as sulfonylureas, meglitinides, metformin, and glitazones do not prevent the progressive decline in insulin secretory capacity (670). Medications that modulate the GLP-1 pathway (GLP-1 receptor agonists and DPP-4 inhibitors) or target the sodium glucose transporter-2 improve glycemic control and have a positive impact on cardiovascular or renal outcomes, but the influence on human islet health and mass are largely unknown. Dual GIP and GLP-1 receptor agonists are also under investigation and have shown encouraging effects on β cell function and weight loss (671, 672).

Importantly, β cell function can be improved, especially early in the T2D course. Bariatric surgery appears to lead to improved β cell function through a mechanism that is not yet determined (673-677). Additionally, intensive dietary interventions (very low calorie or carbohydrate diets), particularly early in the T2D course, can lead to diabetes remission, but only do so when β cell function is restored (414, 678-681). For T2D particularly, defining how such interventions improve β cell function will potentially reveal additional ways to target these pathways.

Maintaining β Cell Mass

An attractive approach has been to protect β cells and promote β cell survival in the face of cytokine, ER, or metabolic stressors that lead to β cell death. There are currently no therapeutics that have been definitively shown to mitigate the deleterious effects of these stress pathways, but numerous targets have shown promise in preclinical or early clinical trials. In T1D, immune modulation through targeting immune cells or signals has shown promise in protecting β cells and slowing β cell loss (682-684). In particular, teplizumab, an anti-CD3 (T cell) antibody, and golimumab, an antitumor necrosis factor α antibody, have shown promising results in phase 2 trials at delaying the onset of clinical T1D or boosting endogenous insulin production, respectively (685, 686). Alternatively, numerous other targets focus on the β cell and seek to modulate its response to such stressors. The calcium channel blocker, verapamil, has been shown to promote β cell survival in patients with recently diagnosed T1D by reducing thioredoxin-interacting protein, which normally promotes apoptosis in β cells (687, 688). Targeting of the vitamin D nuclear receptor appears to promote β cell survival by modulating its response to inflammatory and metabolic signals (689). Histone deacetylase 3 inhibition protects from cytokine-induced β cell death perhaps by preventing transactivation in response to inflammatory signals (690, 691). The anti-inflammatory lipid family palmitic acid esters of hydroxy stearic acids also appear to reduce cytokine-induced ER stress in mice and human islets ex vivo (692). Finally, GLP-1R agonists, already used clinically to boost β cell function, have been proposed to also have a role in reducing β cell ER stress and promoting survival (693, 694). Translating these broad range of pathways to protect endogenous β cell mass to the clinic will be an exciting next avenue in the treatment of diabetes.

Increasing β Cell Mass

Currently, there are 2 general approaches to stimulate endogenous β cell growth in efforts to increase β cell mass: (1) harnessing the mechanisms, growth factors, hormones, and signals involved in normal, physiologic islet growth (development or pregnancy); and (2) identifying small molecules and/or compounds that induce proliferative pathways. Compared to mouse β cells, human β cells are far more resistant to proliferation, which has been a challenge in the field. Nonetheless, intracellular signaling through phosphoinositide-3-kinase, the calcineurin/nuclear factor of activated T cells, and the mechanistic target of rapamycin pathways have been implicated in inducing human islet cell proliferation (537, 538, 695-698). While the machinery for cell cycle progression is largely conserved between humans and rodents, the majority of cyclins and cyclin-dependent kinases in human β cells are sequestered in the cytoplasm rather than the nucleus, possibly explaining the resistance to proliferative signals (699-703). Studies performing high-throughput small-molecule screens have identified candidate molecules such as harmine and 5-iodotubercidin that target the dual-specificity tyrosine-regulated kinase 1a (DYRK1A) to induce β cell proliferation (704, 705). Recently, inhibition of DYRK1A combined with either stimulation of the GLP-1R or with inhibition of transforming growth factor β were shown to be additive in promoting human β cell proliferation (706-708). This is significant because it allows for the use of both agents at lower doses that limit off-target effects, since these pathways are not β cell specific and in the case of DYKR1A, both over and underproduction have been linked to CNS effects (709). Alternatively, cell-specific targeting and active compound delivery, as discussed subsequently, will be essential. Further, new developments with intact human pancreatic slices will aid in our understanding of how these compounds control long-term endocrine regeneration with intact cytoarchitecture (69). Finally, work remains to establish that newly formed β cells via targeting of these pathways are appropriately functional.

Transdifferentiation/Neogenesis

Cellular reprogramming through induced differentiation (neogenesis) or transdifferentiation are exciting concepts to replenish β cell mass, though much work remains to establish this as a viable approach. While multipotent pancreatic progenitors have a clear role in development, there is not yet convincing evidence of a true pancreatic stem cell that could be targeted in the adult human islet or pancreas (710). On the other hand, cellular plasticity of other terminally differentiated cell types has been demonstrated in several mouse models. Models of extreme β cell loss (711, 712) and numerous genetic approaches (713-719) have all led to the creation of rodent insulin-producing cells. While equivalent studies in human islet cells are quite limited, a recent study reported that human islet non-β (mainly α) cells can become insulin producing with exogenous expression of MAFA and PDX1 (81). There is a need for more work to characterize the phenotype of reprogrammed cells in order to understand how similar they are to native human β cells.

Selective Targeting of β Cells or Islets

Importantly, many of the pathways in pancreatic islet biology targeted to improve β cell function, protect β cells, and manipulate β cell growth are also present in other cells, making it unlikely there is a β cell–specific process that can be therapeutically targeted. While certain pathways or targets may be enriched in islet cells and thus inherently targeted, it is more likely that safe and effective therapeutic manipulation of desired pathways will require delivery of the therapeutic compound to islet cells using an engineered carrier (eg, an aptamer, an antibody, a virus). Another challenge for delivery is that the unique macroanatomy and microanatomy of the islet may make targeting and cargo delivery to the β cell difficult. To meet these challenges, numerous groups are working to identify and validate cell surface markers that are specific to or enriched in the β cell, such as NTPDase3 and GLP-1R, or in the α cells, such as HPa3, or in all islet endocrine cells, such as HPi1 (158, 161, 720). In this way, aptamer- or antibody-based systems may allow cell- or islet-specific delivery of a therapeutic agent that would otherwise have broad effects (721). Additional targeting approaches include the use of viruses, primarily adeno-associated viruses, to achieve cell specificity, either through pseudotyping to achieve the desired tropism (722) or through the identification of cell-specific promoters to regulate gene expression of the viral cargo (151, 723, 724). Finally, chimeric antigen receptor T cells (CAR T cells), T cells with genetically engineered artificial T cell receptors, are being used in cancer biology to target specific cell populations and may represent an attractive approach to islet targeting (725, 726). Overall, it is clear that approaches to provide β cell or islet specificity are critical in the development of islet- or β cell–directed therapeutics.

Looking Forward: Topics to Explore Relating to Therapeutic Implications and Clinical Strategies

- How do current therapies (medication, bariatric surgery, weight loss, etc) alter islet cell function and mass and the progression of T2D?
- Are embryonic stem– and induced pluripotent stem–derived insulin-producing cells sufficient for restoring glucose homeostasis, or are additional cell types required?

Conclusions and Looking Forward

This review describes some of the remarkable advances over the past 100 years in our understanding of the human islet, but also reminds us that there is much to learn if we are to prevent or reverse human diabetes-related islet dysfunction or loss. In reviewing prior work, we were struck by DeWitt's introductory statement published in 1906 in "Morphology and Physiology of Areas of Langerhans in Some Vertebrates"—this still rings very true today:

> Probably no organ or tissue of the body has been the subject of more thought or investigation than have the islets of Langerhans, especially during the last few years, and yet there are many questions that still remain unanswered.
>
> -Lydia M. Dewitt, University of Michigan, 1906 (7)

Throughout the preceding sections of this review, we have highlighted topics that merit attention from current and future investigators as we enter the second centennial after insulin's discovery (see prior subsections entitled "Looking Forward"). Some of these processes can be studied in nonhuman systems and this will provide guidance, but the field needs new experimental approaches and tools and access to carefully processed human tissue and cells to better understand the human islet's role in normal physiology and diabetes. Excitingly, numerous resources have been created in recent years with the aim of sharing and integrating data sets (Box 1: Resources for Human Islet Researchers), and these entities will surely help facilitate cross-discipline collaborations offering new insights into human islet biology.

Hopefully, these and many other unanswered questions about the human islet mini-organ will be investigated and answered before the bicentennial observation of insulin's discovery. In this way, diabetes-related islet dysfunction and failure will become an example of how medical and scientific discovery reduced human suffering from diabetes and improved human health.

BOX 1. RESOURCES FOR HUMAN ISLET RESEARCHERS

Human islet functional data

- Alberta Diabetes Institute IsletCore (RRID:SCR_018566); http://www.isletcore.ca
- Human Islet Phenotyping Program (IIDP; RRID:SCR_014387); https://iidp.coh.org/secure/isletavail/

Genetic and epigenetic datasets

- Diabetes Epigenome Atlas (RRID:SCR_016441); http://diabetesepigenome.org/
- T2D Knowledge Portal (RRID:SCR_014533); http://t2d.hugeamp.org/
- TIGER (T2DSystems; RRID:SCR_018913); http://tiger.bsc.es/

Imaging and histology

- Nanotomy (RRID:SCR_018565); http://nanotomy.org/OA/nPOD/
- nPOD Online Pathology (nPOD; RRID:SCR_014641); https://www.jdrfnpod.org/for-investigators/online-pathology-information/
- Pancreatlas (RRID:SCR_018567); http://pancreatlas.org/

Multidimensional data sets

- PANC-DB (HPAP; RRID:SCR_016202); https://hpap.pmacs.upenn.edu/
- Expression and Spatial analysis Pancreas Atlas Consortium Europe (ESPACE); https://www.espace-h2020.eu/
- RHAPSODY, IMI consortium; https://imi-rhapsody.eu/

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