The International Diabetes Federation estimates 415 million people have diabetes worldwide, and this number is expected to increase to 642 million by 2040 (1). These individuals can be segregated into type 1 diabetes (T1D) or type 2 diabetes (T2D) based on etiology of the disease, with T2D accounting for more than 90% of diagnosed individuals. Patients with T1D and long-standing T2D diabetes are generally characterized by dysfunctional regulation of blood glucose coinciding with reduced islet mass and insulin-deficiency (2–4). Both T1D and T2D patients will prematurely develop serious comorbidities, including kidney and vascular complications (5, 6), ultimately reducing quality of life and shortening life expectancy. It is estimated that 12% of global health care expenditures ($673 billion) is spent on diabetes. Thus, development of improved or curative therapies is warranted to combat global insulin deficiency in the growing number of individuals with diabetes. Accordingly, the capacity to study human islet function is essential to enhance our understanding of islet development, genomics, mechanisms of insulin/glucagon secretion, signaling networks, and the pathophysiology of β-cell dysfunction underlying diabetes (7–10).

Historical Perspective on Islet Transplantation: Advancing Our Understanding of Human Islet Biology

In 2000, the pioneering studies conducted by Shapiro et al led to the development of the Edmonton Protocol, and established the concept that insulin-independence could be achieved by the restoration of β-cell mass during T1D (11, 12). Portal vein transplantation of cadaveric human islets immediately improved glucose control and resulted in insulin independence for more than 1 year in brittle T1D patients. Despite aggressive immunosuppressive therapy, transplanted islets were eventually destroyed by allogeneic rejection and underlying autoimmunity; as a result most patients returned to exogenous insulin therapy (13). Unfortunately, widespread application of this approach has been compromised due to an extreme shortage of donor pancreata available for transplantation. On the other hand, harvesting islets for transplantation also increased islet accessibility for basic and preclinical research that has significantly advanced our understanding of human islet biology, and has provided researchers with rational directions to develop novel therapies for diabetes.

Over a decade later, proliferation and insulin production within residual β-cells was observed in the pancreata of individuals who had long-standing T1D for more than or equal to 50 years (medalists) (14). Taken together with knowledge extending from the Edmonton protocol, the concept that sustained and regulated normoglycemia could be achieved through the restoration of β-cell mass became a relevant and feasible target for multiple therapeutic strategies. Research began to shift focus from improving symptomatic treatment to developing curative therapies for patients with both T1D and advanced T2D. Currently under intense preclinical investigation are 2 broad approaches that have demon-

Abbreviations: BMI, body mass index; CIT, cold ischemia time; GSIS, glucose-stimulated insulin secretion; HbA1c, glycated hemoglobin A1c; IEQ, islet equivalent; T1D, type 1 diabetes.
strated promising results in humanized rodent models. The first approach entails the generation of an unlimited supply of exogenous β-cells/islets derived from pluripotent sources, such as human embryonic or induced pluripotent stem cells, for replacement therapies (15–17). Second is the potential to stimulate endogenous islet regeneration in diabetic patients, using the transfer of regenerative cell types (18–22) or stimuli (23). Importantly, human and rodent islets possess key differences in cytoarchitecture (24), neuronal input (25), and electrical machinery regulating β-cell excitability and insulin secretion (26). Thus, recent movement towards clinical application for the transplantation of human embryonic stem cell-derived islets has required careful comparative studies using human islets as the standard. In addition, future development of cell, peptide or drug effectors that expand β-cell mass will continue to rely on preclinical proof-of-concept using human islets.

**Procurement Parameters and Donor Characteristics Relevant to Human Islet Function**

Currently, there exist only a few initiatives that facilitate the isolation and distribution of human islets for preclinical research. These include the Integrated Islet Distribution Program sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, the European Consortium for Islet Transplantation, and several commercial suppliers. Despite the establishment of these programs, the demand for human research-grade islets still outweighs the supply. Escalated costs and reduced availability have made the acquisition of human islets unattainable for many research laboratories (27, 28). Therefore, efforts solely focused on the provision of research-grade human islets would increase availability while reducing or avoiding the financial burden of Good Manufacturing Practice protocols required for transplantation. In addition, human islet isolation has proven highly variable despite standardization of current isolation techniques. Accordingly, islet distribution programs can benefit from high-throughput screening methods to assess how procurement parameters and donor characteristics can affect the yield, purity, and functions of isolated islets (28).

In the February 2016 issue of *Endocrinology*, a team extending the originators of the Edmonton Protocol, describe state-of-the-art human islet preparation specifically for research purposes (29). Focusing on islet purity rather than quantity, Lyon et al (29) obtained 142 donor pancreata over a period of 5 years at the Alberta Diabetes IsletCore Program. Of the 142 pancreata accrued, 100 donors were considered nondiabetic, 7 donors (5%) had T1D, and 24 donors (17%) had diagnosed T2D. This team also carefully documented procurement parameters such as cold ischemia time (CIT) and other donor characteristics such as age, body mass index (BMI) (30), and glycosylated hemoglobin A1c (HbA1c) to correlate with analyses of islet purity and yield. Importantly, the authors also assayed for relevant molecular and functional endpoints in vitro, such as islet insulin content, glucose-stimulated insulin secretion (GSIS), and performed electrophysiological experiments to investigate the dynamics of insulin release at the level of a single β-cell. Although previous studies have attempted to capture many of these parameters using retrospective metaanalyses (31), this study for the first time combines strong statistical power calculations with unique functional analyses performed in a controlled fashion at a single site. Please refer to Table 1 for a general summary of procurement parameters and donor characteristics that were found to affect islet yield and functions.

**Cold Ischemia Time Reduced Islet Yield and Insulin Content but Did Not Impair Insulin Secretion**

CIT, the directed cooling of an organ under reduced or diminished blood supply, has been previously reported to reduce total islet yield (31–36). Lyon et al (29) confirmed total islet equivalent (IEQ) was significantly reduced from pancreata with a CIT more than or equal to 18 hours compared with less than or equal to 12 hours. To assess the effects of extended CIT on isolated human islet functions, the authors used electrochemiluminescence to measure insulin content collected from the supernatant of isolated human islets stimulated under high

**Table 1. Summary of Procurement and Donor Characteristics That Affect Islet Yield or Function**

<table>
<thead>
<tr>
<th>Isolation</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEQ</td>
<td>Purity</td>
</tr>
<tr>
<td>CIT</td>
<td>↓↓</td>
</tr>
<tr>
<td>Culture time</td>
<td>↓↓</td>
</tr>
<tr>
<td>Age</td>
<td>↓↓</td>
</tr>
<tr>
<td>HbA1c</td>
<td>↓↓</td>
</tr>
<tr>
<td>Sex</td>
<td>↓↓</td>
</tr>
<tr>
<td>BMI</td>
<td>↓↓</td>
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</tbody>
</table>

IIC, islet insulin content; Abs GSIS, absolute GSIS; % GSIS, GSIS as a percentage of islet insulin content; SI, stimulation index; CIT, cold ischemia time; HbA1c, glycosylated hemoglobin A1c; BMI, body mass index.
Can Donor Characteristics Explain the Broad Heterogeneity Observed in Isolated Human Islets?

Of the 142 pancreata used in this study, 53.3% and 46.5% were from males or females, respectively. Although the weight of male pancrea was larger than female counterparts, the authors did not observe any statistical significance between males and female donors regarding total islet yield, islet purity, or islet function. Importantly, non-diabetic female donors were found to be significantly older than male counterparts (57.2 vs 51.9 y); as a result the age of nondiabetic donors was investigated to explain the heterogeneity observed among donor islets. In agreement with previous reports (31), a negative correlation was observed between IEQ yield and increasing donor age (Table 1). Surprisingly, islet insulin content was significantly increased in islets harvested from donors more than or equal to 60 years old compared with donors less than or equal to 50 years old (Table 1). Although absolute insulin secretion in vitro did not deviate with donor age, insulin secretion normalized to insulin content was significantly decreased in donors more than or equal to 60 years old, suggestive of modestly impaired insulin secretion. Finally, pancrea from donors with a BMI more than 30 provided larger islets than when collected from donors with a BMI less than or equal to 25. However, the physiological relevance of increased islet size remained elusive, as islet insulin content and GSIS were not affected by donor BMI (Table 1). Collectively, islet yield or function was not affected by donor sex or BMI, and donor age emerged as a relevant consideration impacting islet function. However, the broad range of donor-to-donor heterogeneity could not be completely explained by donor sex, age, or BMI, and prompted the team to assess the impact of HbA1c status on islet function.

Glycated hemoglobin (HbA1c) levels can provide valuable insight into the control of blood glucose within an individual over time, and HbA1c has become a key parameter for diagnosing diabetes (41–45). Prolonged periods of elevated HbA1c significantly contribute to the microvascular complications of diabetes such as retinopathy, neuropathy, and nephropathy. Considering human islets are highly vascularized microstructures (46–49), discrepancies between donor HbA1c levels on isolated islet yield and function was closely examined. Importantly, total IEQ and purity were severely reduced with increased donor HbA1c levels. In addition, insulin content, simulation index, and GSIS of isolated islets at 16.7mM glucose all negatively correlated with increased donor HbA1c levels. Thus, HbA1c levels of donors should be heavily considered during preclinical experimentation using human islets, specifically when assessing insulin production and secretory parameters.

Insights From Diabetic Donor Islets

Direct functional comparison between islets isolated from normal vs diabetic donors represents an extremely valuable opportunity to gain insight into the pathophysiology of T1D and T2D using modern molecular techniques (7). Of the 142 pancreata reported within this study, only 7 donors (5%) were identified as T1D. Al-
though the sample size of T1D donors precluded rigorous statistical analyses, and weak dithizone-staining made the quantification of IEQ yield and purity difficult, the authors were able to isolate islets from all 7 pancreata from donors with T1D. Insulin positive cells were identified in both isolated islets and paired pancreatic tissue samples collected from T1D patients. Moreover, the authors were able to detect insulin secretion in the supernatant of islets from T1D donors, supporting the concept that β-cell content and activity are not completely diminished within T1D patients (14).

Combining the T2D cohort containing 24 donors (17%), with an additional 6 donors (4%) considered undiagnosed T2D due to Hb1Ac levels more than 6.5%, total islet yield and purity from donors with T2D were significantly reduced compared with nondiabetic donors. In advanced T2D patients insulin secretion is often reduced (50, 51) despite decreased insulin sensitivity in peripheral tissues. Indeed, both islet insulin content and GSIS were significantly lower in islets from T2D donors compared with nondiabetic donors. However, a secretory defect was not observed when insulin secretion was normalized to islet insulin content. Collectively, the authors have clearly established the feasibility of obtaining research-grade islets from both T1D and T2D donors. Further studies comparing normal with diseased islets human islets will provide a valuable tool to advance our understanding of the pathophysiology that underlies the spectrum of diabetes. In addition, human islets from diabetic donors could potentially be used for new pharmaceutical testing for T2D therapy.

Conclusion and Future Directions

The Alberta Diabetes IsletCore was created for the isolation, banking, and distribution of research-grade islets from donors that were declined for clinical transplantation. According to the IsletCore website (www.bcell.org/isletcore), this program has isolated 30 million IEQ, distributed 12 million IEQ, and currently have over 5 million IEQ cryopreserved. With widespread distribution the potential impact of such a program towards the progression of human islet research is immense. Because the demand for human islets currently exceeds the supply, the average wait time to obtain human islets from national programs is at minimal 2 weeks, thus a more prolific source of research-grade human islets is expected to increase the rate of human β-cell research by at least 2-fold (28). Accordingly, it is critical that programs such as IsletCore determine whether relaxing criteria for acceptable donor or isolation characteristics would increase availability of research-grade islets. Because the number of islets required for research is much lower than those needed for transplantation procedures, it is wise to collect human islets from pancreata that do not meet the criteria for clinical transplantation but remain functionally intact.

The work by Lyon et al (29) provides valuable insight regarding the feasibility and limitations of research-focused islet isolation, distribution, and banking (29). This manuscript identifies parameters of procurement, such as CIT, which negatively impacted islet yield but did not significantly impair islet secretory function in in vitro analysis. In addition, donor characteristics such as age or HbA1c levels were found to account for some of the functional heterogeneity observed among human donor islets, whereas parameters such as sex or BMI were less important. Taken together, the authors have established standards for research-grade islets; in return establishing a foundation to build upon in the future.

The translation of preclinical research towards clinical application will require convincing functional evidence obtained from studies using human islets. Thus, future research aimed at better understanding human islet physiology should focus on in vitro and in vivo experimentation with isolated human islets from healthy and diabetic donors. Collectively, this study provides a template to develop programs whose focus is to provide means to isolate, cryopreserve, and distribute research-grade human islets in order to fulfill the current void within preclinical research. Increasing the availability and equitable access to human islets will help propel our understanding of islet physiology and pathophysiology, and will facilitate development of novel drug, peptide or cell-based therapies for diabetes mellitus.

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