Islets for Research: Nothing Is Perfect, but We Can Do Better

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In December 2018, *Diabetes* and *Diabetologia* began requiring authors of papers reporting data obtained from studies on human islets to report critical characteristics of the human islets used for research. The islet community was asked to provide feedback on it. Here is the contribution by the European Consortium for Islet Transplantation.

A call for improved reporting of human islet characteristics in research articles was recently launched by the editorial boards of *Diabetes* and *Diabetologia* (1,2). The call came from a review by Hart and Powers (3) that identifies as challenges associated with human islet studies the wide functional heterogeneity observed between islet preparations and the highly variable (and often inadequate) reporting of human islet characteristics in the scientific literature. As first authors will be required to provide, in table form, the source, isolation center, and unique identifier number for each islet preparation, the age, sex, BMI, and HbA_{1c} (or other measures of glucose control) of the donor, and whether she or he had diabetes or not, feedback from the islet community was requested to address the issue.

The European Consortium for Islet Transplantation (ECIT) will be very happy to adhere to this request and to complete the form that describes, as best as possible, donor and islet preparation characteristics. Since 2006, ECIT has decided to start the human islet distribution program for basic research, Islet for Research, for the shipping of high-quality human islets to the European diabetes research community in order to advance scientific discovery and translational medicine. ECIT involves the San Raffaele Scientific Institute as coordinator (Milan, Italy), the Hôpitaux Universitaires de Genève (Geneva, Switzerland), the Uppsala University Hospital (Uppsala, Sweden), and the CHU de Lille. ECIT is supported by JDRF International.

A web-based platform was developed in 2009 to coordinate and track program activity (http://ecit.drisanraffaele.org/en/register/index.html). Considering the last 32-month period (from 1 January 2016 to 31 August 2018), as the activity of the previous 5 years has already been published (4), 172 research groups have completed the online registration form and received approval to submit an islets request to the ECIT consortium. Of these, 49 research groups have filled at least 1 request to receive islets and a total of 64 requests were submitted from 13 European countries. Considering individual requests, the median number of requested islets was 20×10^3 (IQR 25.6×10^3) in 20 (IQR 30) shipments. The total number of islets requested was 57.987 million. An ideal islet purity ≥70% was requested in 97% of the applications. During the 32 months of the program (January 2016 to August 2018), 18.778 million human islets were distributed throughout 571 shipments. The production and delivery of islets by the ECIT centers have been stable over the years. However, because of the burgeoning demand for islets and the lack of funding to cover additional islet isolation procedures, the ECIT centers went from supplying 74% of the requested islets in 2010 (4) to only 32% in the last period.

The mean perceived quality feedback from the users scored 1.84 ± 0.7 (mean range 1.67–2.08 per center) in a scale of 1 to 4 (1 = excellent, 2 = good, 3 = fair, 4 = bad), and 90% of the islets processed and distributed by ECIT

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were ranked as excellent or good in quality. A total of 49 academic investigators representing 37 institutions received human islets for 52 approved studies. These studies have so far resulted in 71 publications (January 2016 to August 2018) directly attributable to the use of human islets supplied by ECIT laboratories. An Institute for Scientific Information Web of Knowledge search in September 2018 yielded information on 69 of these citations, with an average impact factor per item of 6.53, showing that articles appeared in more than 37 journals, with a median impact factor of 5.363, and were cited 476 times from 2016 to 2018 (average citations per item, 6.9; average citations per year, 119; citations from 2017, 227; H index, 10). From this experience and from similar islet distribution programs, it is evident that sufficient supply and further stimulation of translational research is critically dependent on enhanced availability of high-quality human islet preparations.

As the call asked the islet community to provide feedback to help to refine and further expand the checklist (with the hypothesis that also functional or other parameters were required to be assessed), we would like to give our contribution. Even if it is possible to further document and provide functional and/or composition parameters of human islet preparations, we want to underline some risks and related problems. First, the reliability of some information is debatable in the context of brain death donors. Obviously, data such as sex and age are indisputable, but even simple data, such as weight and height, can be approximate, as can some anamnestic information (duration and history of diabetes, family history of diabetes, diet, and lifestyle, alcohol consumption, etc.). Moreover, the biochemical data, such as glucose and HbA_{1c}, can be heavily influenced by the treatment in the critical care unit (glucose infusion, steroids, insulin, transfusions [5], etc.).

Second, the wide functional heterogeneity observed between islet preparations is an intrinsic property and an unsolved issue for any human primary cell. It is well known that multiple physiological (e.g., nutritional status, hormone levels, lifestyle, etc.), pathological (cause of death, presence of infections/inflammation, treatment, etc.), and genetic factors may influence the number, the composition, and the function of human pancreatic islets. To obtain more consistency in the data, it would perhaps be better to request a minimum number of subjects studied. Although the use of different donors leads to increased variance, the strength of reproducing results with cells from several donors will support the generality of the results.

Third, despite the automated method described by Ricordi et al. (6) that represents the backbone of islet isolation in all facilities, every center optimizes and standardizes its own procedures and processes within the center's unique framework of regulatory issues, donor organ availability, and quality, local processing facility requirements, and financial considerations (7). The intricate nature of pancreatic islets and the limited number of reliable characterization assays for assessing the quality of the islets obtained make the standardization of the process complicated. For example, the current metrics used to qualify human islet preparations are rather loose. Heterogeneity in the methods to count islet equivalents is vast, including real count or an estimate based on pellet volume and purity. A promising tool to overcome this problem, called IsletNet, was recently presented by David Habart ("IsletNet: an online service for standardized islet counting" at the 9th EPITA [European Pancreas and Islet Transplant Association] Symposium and 38th AIDPIT [Artificial Insulin Delivery, Pancreas and Islet Transplantation] Workshop, Igls, Austria, 27–29 January 2019). IsletNet is an online service for standardized islet counting (https://isletnet.com/) developed in collaboration with several of the high-volume islet isolation centers in Europe: it appears fast, accurate, and a much awaited user-friendly technology. This tool is not yet routinely used, but it could be implemented by islet community and grant agencies. Moreover, the use of different shipping methods, such as commercial airline transporters, chartered jet, or vehicles, based on the distances, can add further to the level of heterogeneity.

Fourth, to improve the consistency of results, we highlight the importance of standard handling of islets after isolation and shipment, a variable that could be more important than donor-related factors, intensive care unit stay, and islet isolation techniques. Some researchers receiving islet shipments have no proper training with human islets and ignore some of basic specific needs, including culture density according to purity, frequency of media renewal, and gentle manipulation to avoid mechanical stress on islets. This suboptimal handling by researchers of human islets after the shipment could severely impair and further aggravate the functional capacity of the islets used in their experiments. It may be suggested that researchers receiving human islet shipments are required to have some training and/or that isolation centers provide technical recommendations about culture conditions upon arrival. A yearly "workshop" associated with the European Association for the Study of Diabetes could be a good suggestion to implement the expertise of the European users. Alternatively, some technically demanding experiments could be preferably performed directly in the human islet isolation laboratory. One recommendation would be that all experimental studies with shipped human islets should start with a "run-in"/quarantine period where only islet preparations that pass predefined "release criteria" tests should be entered into a team's specific experimental studies.

Fifth, we have to be cognizant that any additional data or biological tests applied for the standardization of human islet for research will involve an increase in costs. The information suggested by the journal editorial boards can be added at low cost, but other actions, such as the centralization of islet "phenotyping," cannot be obtained in the absence of relevant additional resources. This could

be performed in some contexts, such as the Integrated Islet Distribution Program, thanks to dedicated resources made available by the National Institutes of Health. Other contexts are different. In Europe, there is no supranational government agency that can act as the National Institutes of Health does in the U.S. ECIT is in place only thanks to the extraordinary help of JDRF, with a budget that has not increased in the past 13 years despite increased production costs; however, the ECIT budget is expected to be reduced from 2020 on. In addition, in many European centers, islets can be used for research only if they are not usable for the clinic (7). This means that the production must be performed according to expensive clinical-grade processing regulations (Current Good Manufacturing Practices). We have to be aware of the risk of adopting an expensive standard of characterization in the absence of resources because it could drastically reduce the availability of islets or push the field into the commercial arena, which would mean no chance for approval by ethics committees/institutional review boards in many European Union countries and very high costs.

In conclusion, human pancreatic tissue and isolated islets of Langerhans are a vital resource for both type 1 diabetes and type 2 diabetes research. Given recent progress in stem cell biology (8), islet genomics (9,10), potential novel therapeutic approaches (11), and many other advances, access to human islets for research is critical (12). Human pancreatic islets remain the gold standard for the assessment of β-cell function, despite a variety of surrogate alternatives, including nonhuman pancreatic islets or rodent/human β-cell lines (13–16). Any action taken to improve the field is more than welcome, but we must be careful to avoid following models that, beyond good intentions, could jeopardize the possibility of studying this precious tissue.

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