Original Article

MOLECULAR METABOLISM

# Individual islet respirometry reveals functional diversity within the islet population of mice and human donors

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## ABSTRACT

**Objective:** Islets from the same pancreas show remarkable variability in glucose sensitivity. While mitochondrial respiration is essential for glucose-stimulated insulin secretion, little is known regarding heterogeneity in mitochondrial function at the individual islet level. This is due in part to a lack of high-throughput and non-invasive methods for detecting single islet function.

**Methods:** We have developed a novel non-invasive, high-throughput methodology capable of assessing mitochondrial respiration in large-sized individual islets using the XF96 analyzer (Agilent Technologies).

**Results:** By increasing measurement sensitivity, we have reduced the minimal size of mouse and human islets needed to assess mitochondrial respiration to single large islets of $>35{,}000\ \mu m^2$ area ($\sim 210\ \mu m$ diameter). In addition, we have measured heterogeneous glucose-stimulated mitochondrial respiration among individual human and mouse islets from the same pancreas, allowing population analyses of islet mitochondrial function for the first time.

**Conclusions:** We have developed a novel methodology capable of analyzing mitochondrial function in large-sized individual islets. By highlighting islet functional heterogeneity, we hope this methodology can significantly advance islet research.



**Keywords** Islets; Mitochondria; Respirometry; Glucose

## 1. INTRODUCTION

Pancreatic islets rely on mitochondrial respiration to secrete insulin [1], a critical function for maintaining metabolic homeostasis. A rise in extracellular glucose levels increases TCA cycle flux, mitochondrial respiration, and ATP synthesis in islet β-cells, which generates molecular signals stimulating and amplifying insulin secretion [2]. Indeed, even at the level of the whole organism, mitochondrial respiratory function accounts for approximately 90% of total oxygen consumption, 80% of which is coupled to ATP synthesis [3]. Therefore, measuring oxygen consumption represents the gold standard assessment of metabolic behavior, bioenergetic demand, and mitochondrial function for islet tissue. Furthermore, by using different compounds targeting mitochondria, one can determine respiration linked to mitochondrial ATP synthesis and the maximal capacity of cells oxidizing fuels through the electron transport chain [4].

Islets and β-cells within an islet are known to show remarkable variability in glucose sensitivity [5–10], size [11,12], architecture [8], and cell composition [13] among other factors. A small group of "first responder" islets rapidly respond to glucose *in vivo* by releasing almost their entire load of insulin, while some islets remain dormant and remain unresponsive to glucose [7,14]. Islet heterogeneity also seems to play a critical role in pathogenesis of metabolic disease, as distinct groups of islets are more susceptible to dysfunction in diabetes [13]. In addition, one could predict that islet preparations with a low number of "responder islets" and a high number of "dormant islets" would be

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*Abbreviations:* OCR, oxygen consumption rate; Ant A, Antimycin A; Oligo, Oligomycin A; FCCP, Carbonyl cyanide-*4*-(trifluoromethoxy)phenylhydrazone

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less likely to efficiently sustain insulin production after their transplantation into subjects with diabetes. This could be the case since a high proportion of islets do not survive transplantation, and a low number of responder islets would decrease the probability of providing sufficient insulin in response to glucose. Despite the essential role of mitochondrial respiration in insulin secretion [1,15], very little is known about heterogeneity in mitochondrial function among islets from a given population. Currently, it has not been possible to address this issue due to a lack of high-throughput methods capable of quantifying mitochondrial function of individual islets within a population.

The current gold standard for islet respirometry is the XF24 islet capture plate [16]. Despite being the most sensitive oxygen consumption methodology, the islet capture plate requires 50–80 islets per well, which is almost the entire islet population from one mouse when performed in triplicate. Furthermore, only 20 different conditions can be tested simultaneously. This experimental platform is not suitable for assessing large numbers of biological samples and lacks the sensitivity to detect respiration of individual islets from heterogeneous populations. Other more invasive methodologies can measure oxygen consumption in single islets [17] but require complex microfluidics chips for encapsulating islets after staining with a fluorescent dye. Single islet ATP measurements are possible [18] but require viral transduction of biosensors. These more invasive manipulations have not been developed for high-throughput measurements, potentially limit the detection of endogenous functional heterogeneity, and could preclude islet use for transplantation. Therefore, a rapid, non-invasive, real-time and high-throughput method is needed to quantify islet functional heterogeneity.

In the present study, we established a non-invasive methodology that enables high-throughput measurement of oxygen consumption in large-sized individual islets from mice and humans. We have developed in collaboration with Agilent Technologies a specialized microplate for the XF instrument called the “spheroid plate”. This plate contains a perfusion insert in each well that allows stable media flow across islets during mixing, without perturbing islet function. The spheroid plate reduces the biological sample size required for mitochondrial respiratory analysis and maintains the functionality for bioenergetics measurements currently used with the XF24 islet capture plate. Our new approach allows a populational analysis of metabolic function that revealed heterogeneous glucose sensitivity in large individual islets from both humans and mice. We hope our methodology can accelerate islet research and help elucidate mechanisms underlying the metabolic heterogeneity in pancreatic islets.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Collagenase P, D-glucose, L-leucine, L-glutamine, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP), and Antimycin A were purchased from Sigma Aldrich (St. Louis, MO). Fatty acid-free bovine serum albumin (BSA) was purchased from EMD Millipore (Billerica, MA). Fetal bovine serum (FBS) was obtained from Life Technologies (Carlsbad, CA). Oligomycin A was obtained from Calbiochem (San Diego, CA). Accutase was purchased from Thermo Fisher Scientific (Roskilde, Denmark). Seahorse XF96 spheroid microplates, Seahorse XF96 FluxPaks, Seahorse XF Calibrant Solution and Seahorse XF Base Medium Minimal DMEM were acquired from Agilent Technologies (Santa Clara, CA).

### 2.2. Isolation and culture of mouse islets

Islets were isolated from 11 to 16 week old male C57BL/6J mice (Jackson Labs, Bar Harbor, ME) via collagenase P injection into the bile duct, as previously described [16,19]. Islets were cultured overnight at 37 °C 5% $CO_2$ in islet media (11 mM glucose RPMI 1640 + 10% FBS + 100 U/mL penicillin, and 100 μg/mL streptomycin) prior to experimentation.

### 2.3. Human islets

Human islets were obtained from the University of Alberta Diabetes Institute Islet Core (Edmonton, Alberta, Canada) in collaboration with Dr. Patrick MacDonald. Islets were isolated from six non-diabetic deceased donors ages 18–71 with 60–95% purity, cultured 7–40 h post-isolation and shipped overnight at 4 °C in CMRL media (Gibco/ThermoFisher, Waltham, MA). Upon arrival, islets were further purified from exocrine pancreas and cellular debris by visual inspection and picking. Islets were cultured overnight in fresh CMRL media at 37 °C and 5% $CO_2$ before measurement of mitochondrial respiration. Human islets were also obtained from the University of Minnesota Schulze Diabetes Institute (Minneapolis, MN, USA) in collaboration with Josh Wilhelm. Islets were isolated from living donors with pancreatitis undergoing total pancreatectomy with islet auto-transplantation (Patient 1: 57 years old, non-diabetic female, BMI = 25, HbA1c = 4.8%; Patient 2: 57 years old, non-diabetic male with hyperlipidemia, BMI = 23, HbA1C = 5.8%). Islets were shipped overnight (4 °C, with Cryopaks) in Transplant Media (CMRL supplemented with 2.5% human serum albumin, 25 mM HEPES and 20 μg/mL ciprofloxacin). Upon arrival, islets were further purified from exocrine pancreas and cellular debris by visual inspection and picking. Islets were then cultured for 1–2 h at 37 °C 5% $CO_2$ in CMRL media supplemented with 10 mM niacinamide, 1% (v/v) insulin-transferrin-selenium, 16.7 μM $ZnSO_4$, 5 mM sodium pyruvate, 1% (v/v) Glutamax, 25 mM HEPES, 10% (v/v) FBS, and 1% (v/v) pen/strep. Respirometry was conducted on the same day that the islets arrived at UCLA. Approximately 1–2% of the final isolated islet product from pancreatitis patients was used for experiments.

### 2.4. XF96 spheroid plate islet respirometry

Mouse or human islets (1–32 islets/well) were seeded into wells of a poly-D-lysine-coated (100 μg/mL) XF96 spheroid plate containing 100–175μL/well of warm assay medium (Seahorse XF base medium minimal DMEM, supplemented with 3 mM glucose and 0.1% FBS). Islet seeding was done by aspirating islets in a minimal volume of media (~4–15 μL) and inserting the pipette tip into each well of the spheroid plate, using a Leica S6E microscope to orient the pipette tip directly over the central depression in the well. Islets fall out of the pipette tip by gravity and into the central detent of each well. Once seeded, islets in the plate were centrifuged at 450 rpm for 7 min with no centrifuge brake, then incubated for 1–2 h at 37 °C in a non-$CO_2$ incubator. Mitochondrial respiration was measured using the Seahorse XF96 extracellular flux analyzer equipped with a spheroid plate-compatible thermal tray (Agilent Technologies, Santa Clara, CA). Basal respiration was first measured in 3 mM glucose media. Islets were then sequentially exposed to glucose (final concentration in well of 20 mM), Oligomycin A (3.5–4.5 μM final concentration), FCCP (1 μM final concentration) and Antimycin A (Ant A, 2.5 μM final concentration).

To validate individual islet respirometry with the XF96 spheroid plate, individual mouse islets were exposed to either 20 mM glucose or glucose + amino acids (AA, 10 mM each of leucine and glutamine), followed by FCCP (final concentration of 1 μM) and Antimycin A (final concentration of 3 μM). For assays testing reproducibility, single islets were seeded in a spheroid plate as described above, and basal respiration in 3 mM glucose media was measured until steady state respiration was achieved. Islets were then transferred to different wells in a new spheroid plate and steady state basal respiration was

 