



Novel islet respirometry assay reveals high levels of uncoupled respiration in islets

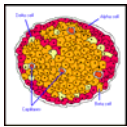
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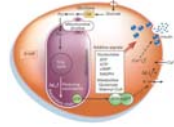


Introduction

Type 2 diabetes presents when the pancreatic islets can no longer secrete sufficient amounts of insulin. A growing body of evidence suggests mitochondrial dysfunction to be central in the pathology of beta cell failure. Mitochondrial metabolism is essential for proper insulin secretion as oxidative phosphorylation produces the majority of the cells ATP, which is required for insulin granule exocytosis. However, mitochondrial substrate oxidation is not fully coupled to ATP synthesis as part of the proton gradient across the inner mitochondrial membrane reenters the matrix through other ways than ATP synthase; this is termed uncoupled respiration or proton leak. In theory, mitochondria with highly coupled oxidative phosphorylation may be expected to produce more ATP and contribute more to insulin secretion. The basic characteristics of islet mitochondrial uncoupled respiration are unknown. In this study we sought to characterize the basal proton leak of intact islets, its regulation as well as its profile in diseased islets.



Islets of Langerhans consists mainly of beta cells



Mitochondrial metabolism is central in the insulin secretory pathway

Methods and materials

XF24 (Extracellular flux analyzer) is designed for measuring oxygen consumption and extracellular acidification from monolayer adherent cells. Islets are non adherent and have a diameter of up to 250 µm. In order to adapt the technology, a plate was developed that immobilizes islets in order to allow reliable measurements (Fig 1).

In the respiratory chain oxygen is consumed at complex IV, which results in proton extrusion from the matrix. Proton extrusion also occurs in Complex I and III, however, oxygen is not consumed. Oxygen consumption, the golden standard for mitochondrial function, both reflects protons used for ATP production (coupled respiration) as well as proton leak (uncoupled respiration). In isolated mitochondria the uncoupled respiration may be estimated by comparing state 3 (excess of ADP) with state 4 (lack of ADP). Since ADP is cell impermeable and ATP depletion is a major cell stressor, the same approach is not applicable to intact cells. Instead, pharmacological inhibitors of mitochondrial complexes may be used to estimate the proton leak / uncoupled respiration. Moreover, the islets of Langerhans are scarce and isolating sufficient amounts of mitochondria from these is not achievable.

Mouse pancreatic islets were isolated from 10-12 weeks old C57Bl6 male mice. At the day of the experiment islets were transferred to DMEM media with 3mM glucose and 1% FBS. 70-80 islets were seeded by concentration in each well of the islet plate, and allowed to equilibrate to the low glucose for 1h at 37°C before loaded into the XF24. OCR was measured at low and high fuel levels as well as under drugs acting on the respiratory chain (oligomycin, FCCP, rotenone, myxothiazol/antimycin A).

Fig 1. Islet plate development

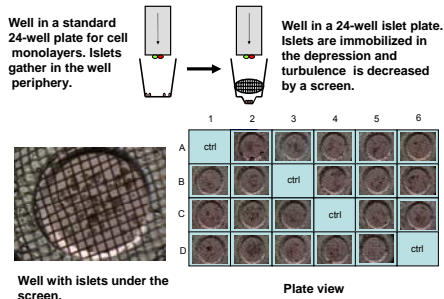


Fig 2. Measuring coupled and uncoupled respiration

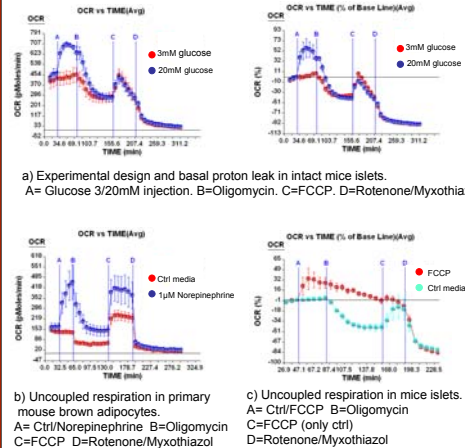
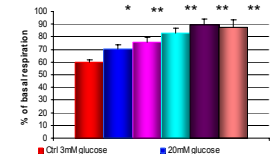
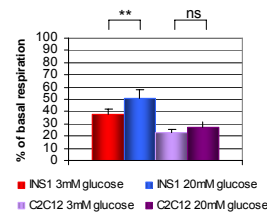


Fig 3. Fuels that stimulate insulin secretion increase islet uncoupled respiration

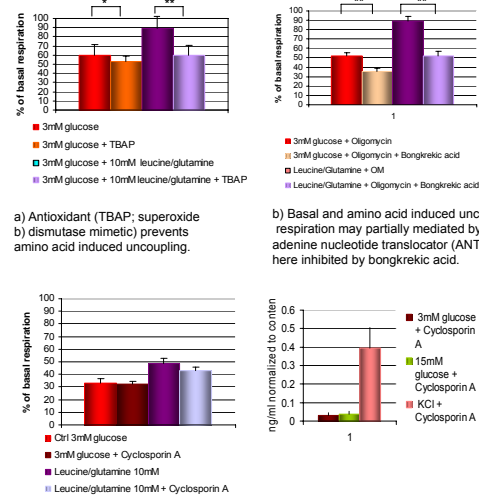


a) Steady state respiration rates under oligomycin are shown; normalized to initial respiration rates.

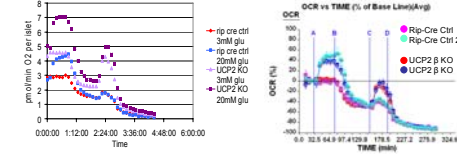


b) For comparison, similar derived data from INS1 beta cells and C2C12 myoblasts.

Fig 4. Mechanisms of uncoupled respiration in islets

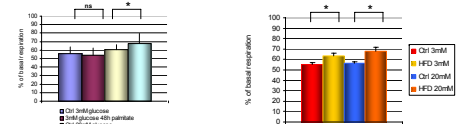


c) Permeability transition pore inhibitor Cyclosporin A has no effect on islet uncoupled Respiration (under oligomycin) but inhibits insulin secretion as expected



d) Beta cell specific uncoupling protein 2 (UCP2) knock out islets exhibit higher basal and FCCP stimulated respiration, however have normal levels of uncoupled respiration. A= Ctr/FCCP B=Oligomycin C=FCCP (only ctrl) D=Rotenone/Myxothiazol

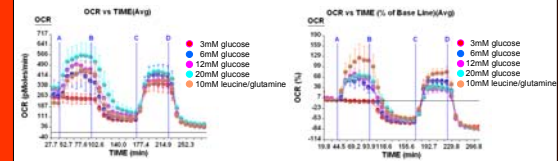
Fig 5. Chronic palmitate and High-fat diet uncouples islet respiration



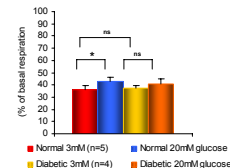
a) 48hour 0.4mM palmitate incubation increase uncoupled respiration (under oligomycin) under high glucose.

b) Islets from high-fat diet-fed mice exhibit higher levels of uncoupled respiration (under oligomycin)

Fig 7. Human islets respirometry



a) Typical respirometry response of normal human islets to glucose and amino acids leucine/glutamine (A), followed by oligomycin (B), FCCP (C) and rotenone/myxothiazol (D).



b) Normal human islets (n=5) show increased uncoupled respiration (under oligomycin) at high glucose while diabetic islets do not (n=4; HbA1c >6%). There is no significant difference in uncoupled respiration between normal and diabetic islets.

Conclusions

To date, assays for islet respirometry are user-dependent or only allows limited number of samples. To enable this study we developed a user-friendly and high-throughput approach based on the XF24 platform. With this method we can concurrently run up to 24 islet samples and test multiple conditions.

By applying drugs acting on the respiratory chain we can estimate the level of fuel-stimulated, uncoupled, maximal as well as non-mitochondrial respiration under various conditions. This approach's specificity for measuring proton leak was confirmed in brown adipocytes and in islets treated with an uncoupler prior to oligomycin inhibition of coupled respiration.

When stimulated with fuels such as glucose mouse islets displayed markedly increases in respiration. The basal level of islet uncoupled respiration was measured to 55%, strikingly higher than other cell types such as C2C12 myoblasts (20-25%). We found that the cellular fuels such as amino acids, free fatty acids and glucose significantly uncouple islet mitochondria and that this is prevented by antioxidants. Further we found that the adenine nucleotide transporter (ANT), but not permeability transition pore (PTP), makes a significant contribution to the proton leak under resting conditions. In vitro incubation with palmitate did not affect the level of uncoupled respiration under low glucose, but had a significant effect when the islets were stimulated with high glucose.

Islets with beta-cell specific deficiency in uncoupling protein 2 (UCP2) were found to exhibit higher basal respiration, however they exhibited normal levels of uncoupled respiration pointing to UCP2 not primarily acting as an uncoupling protein. In addition to the studies on mouse islets, human islets from healthy as well as diabetic donors were tested.

We found that human islets also display high levels of uncoupled respiration that are increased by high glucose. We are currently expanding our cohort of human islets in order to make a comprehensive comparison of normal and diabetic human islets.