

# Islet respiratory leak is acutely regulated by nutrients and ROS and is dysregulated in the obese state

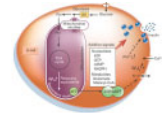
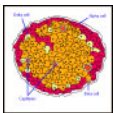
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## Introduction

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. A growing body of evidence suggests mitochondrial dysfunction to be central in the pathology of beta cell failure. However the precise bioenergetic nature of this dysfunction is still unclear.

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. The basic characteristics of islet mitochondrial uncoupled respiration are unknown. In this study we sought to characterize the basal proton leak of intact islets, it's 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 (Extraocellular 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  $\mu$ m. 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 C57B6 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

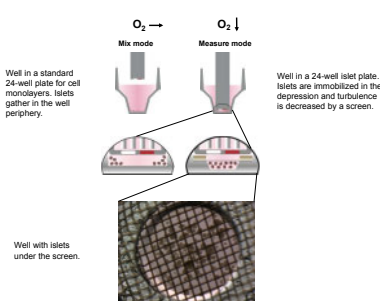
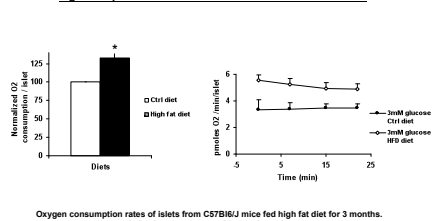


Fig 2. Respiration increased in HFD diabetic mouse islets



Oxygen consumption rates of islets from C57B6/J mice fed high fat diet for 3 months. Animals were obese and hyperglycemic.

Fig 3. Measuring coupled and uncoupled respiration

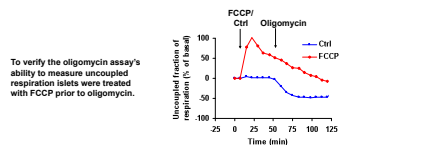
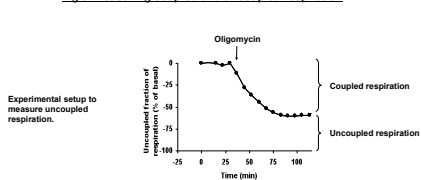


Fig 4. HFD and chronic palmitate uncouples islet respiration

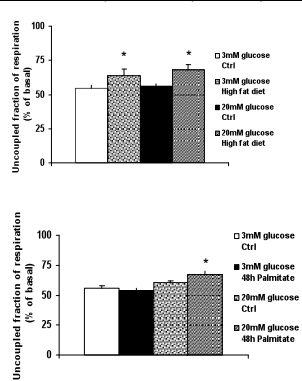
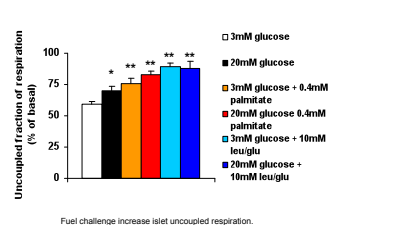
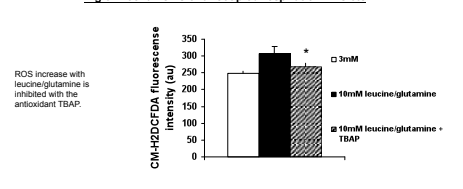


Fig 5. Fuels that stimulate insulin secretion increase uncoupled respiration

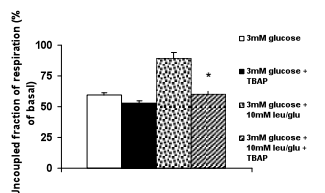


Fuel challenge increase islet uncoupled respiration.

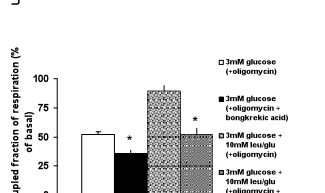
Fig 6. Mechanisms of uncoupled respiration in islets



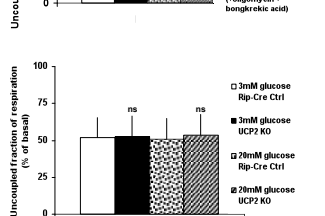
ROS increase with leucine:glutamine is inhibited with the antioxidant TBAP.



Antioxidant TBAP prevents leucine:glutamine induced increase in uncoupled respiration.

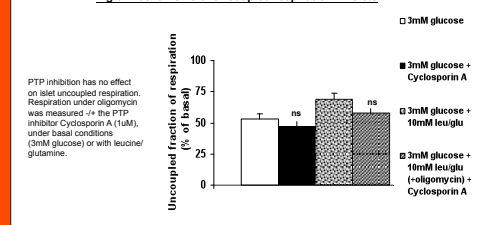


Uncoupled respiration partially mediated by ANT. Respiration under oligomycin was measured +/- the adenine-nucleotide-translocase inhibitor bongkercic acid.



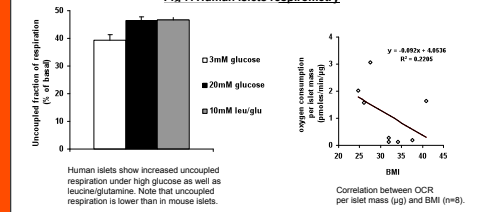
UCP2 play no role in islet uncoupled respiration.  $\beta$ -cell specific UCP2 KO islets vs. Rip-Cre control islets.

Fig 6. Mechanisms of uncoupled respiration in islets



PTP inhibition has no effect on islet uncoupled respiration. Respiration under oligomycin was measured +/- the PTP inhibitor Cyclosporin A (1  $\mu$ M), under basal conditions (3mM glucose) or with leucine/glutamine.

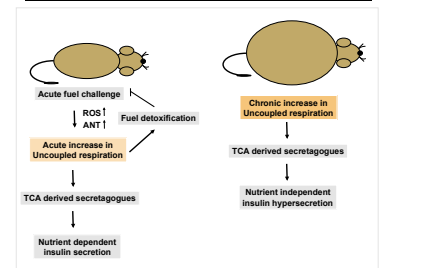
Fig 7. Human islets respirometry



Human islets show increased uncoupled respiration under high glucose as well as leucine:glutamine. Note that uncoupled respiration is lower than in mouse islets.

Correlation between OCR per islet mass ( $\mu$ g) and BMI (n=9).

Fig 8. Possible roles of uncoupled respiration in islet physiology



## Results:

Islets from obese animals exhibit lower respiratory chain efficiency, measured as an increased level of respiration that is uncoupled to ATP synthesis (respiratory leak). Fuels that stimulate insulin secretion acutely increased the respiratory leak. The respiratory leak was completely abolished by treatment with a catalytic antioxidant. Pharmacological analysis revealed that adenine-nucleotide-translocase contributes one-third of the leak while uncoupling-protein-2 and permeability-transition-pore appear not to contribute. Finally, we examined a cohort of human islets and found that human islets exhibited a lower level of respiratory leak. Nevertheless, similar to the mice islet, this leak could be increased by exposure to nutrients.

## Conclusions:

Islet respiratory leak is a nutrient-, ANT- and ROS-dependent mechanism that becomes dysregulated in the obese state. The nutrient dependent increase in the respiratory leak may serve to further increase turnover of the TCA cycle and augment secretion. Altogether, these data suggest a potential new mechanism by which ROS stimulates and obesity dysregulates insulin secretion.

## Acknowledgements:

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