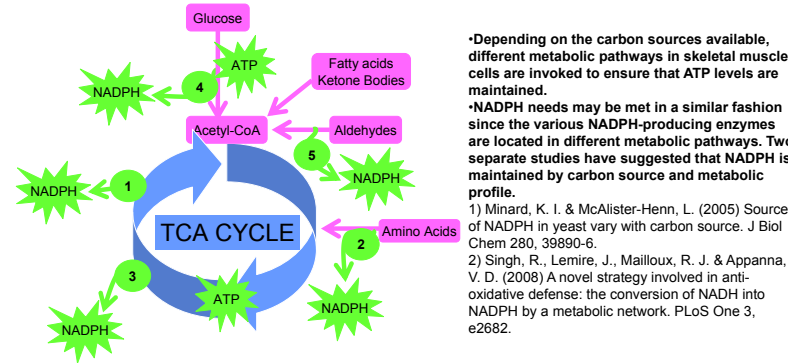


Background

Mitochondria are reliant upon a steady supply of NADPH to support 1) Anti-oxidative defense, 2) Lipogenesis, 3) Redox balance, and 4) Mitochondrial DNA maintenance

NADPH-producing enzymes are located in various metabolic networks. 1) Malic enzyme (PYRUVATE CYCLE), 2) Glutamate dehydrogenase (AMINO ACID METABOLISM), 3) Isocitrate dehydrogenase (TCA CYCLE), 4) Glucose-6-phosphate dehydrogenase/6-phosphogluconate dehydrogenase (PENTOSE PHOSPHATE SHUNT), 5) Aldehyde Dehydrogenase (ALDEHYDE METABOLISM)

Pyridine nucleotides (NADH kinase) and proton gradient (transhydrogenase) also support NADPH pools.

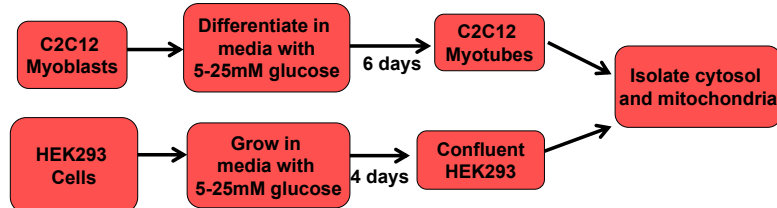


Hypothesis

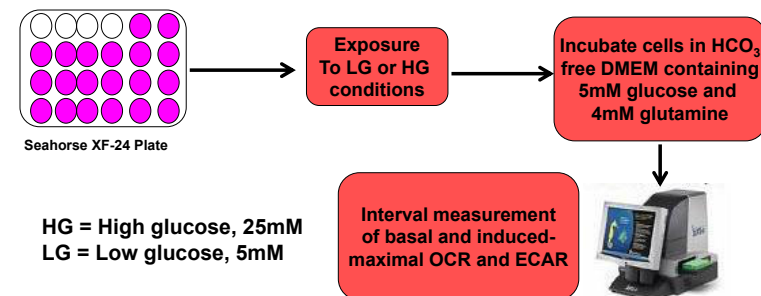
Sources of NADPH in mitochondria are regulated by the metabolic state of the cell and which carbon source is being preferentially metabolized.

Study Design

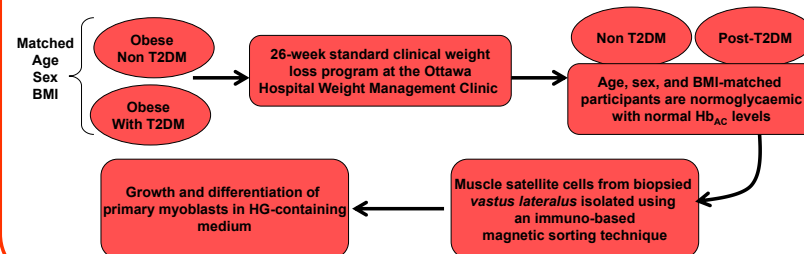
1. Cell culture models



2. In situ analysis of Oxygen Consumption Rates (OCR) and Extracellular Acidification Rates (ECAR) using the Seahorse XF-24 Analyzer

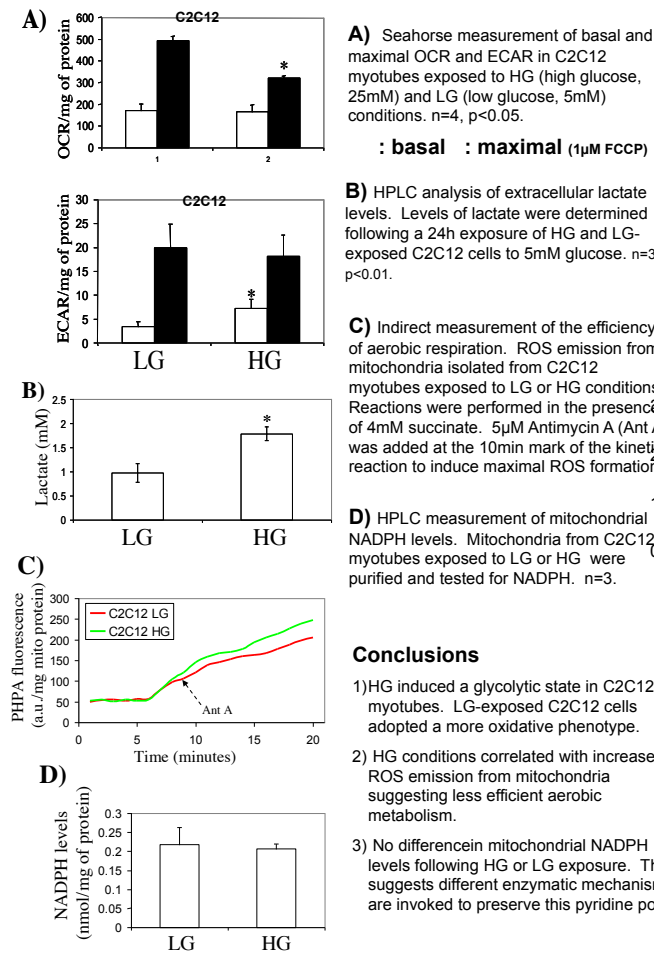


3. Primary human myoblast purification and differentiation.



Results

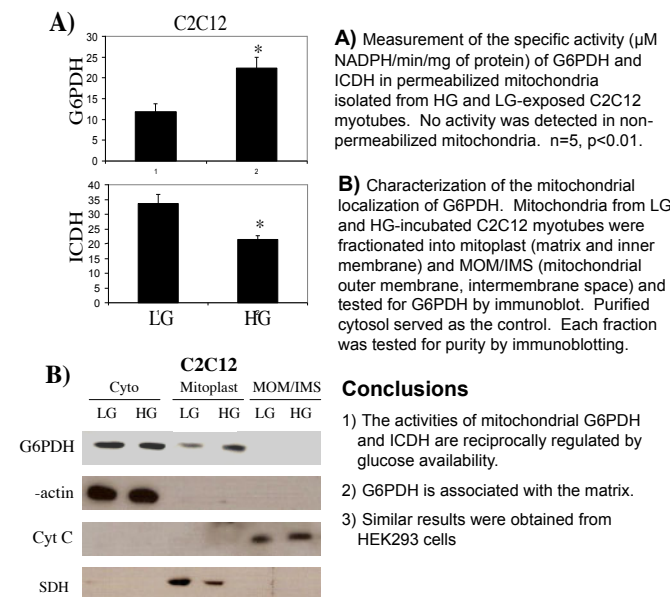
Figure 1: Metabolic Characteristics



Conclusions

- 1) HG induced a glycolytic state in C2C12 myotubes. LG-exposed C2C12 cells adopted a more oxidative phenotype.
- 2) HG conditions correlated with increased ROS emission from mitochondria suggesting less efficient aerobic metabolism.
- 3) No difference in mitochondrial NADPH levels following HG or LG exposure. This suggests different enzymatic mechanisms are invoked to preserve this pyridine pool.

Figure 2: Identification of mitochondrial G6PDH

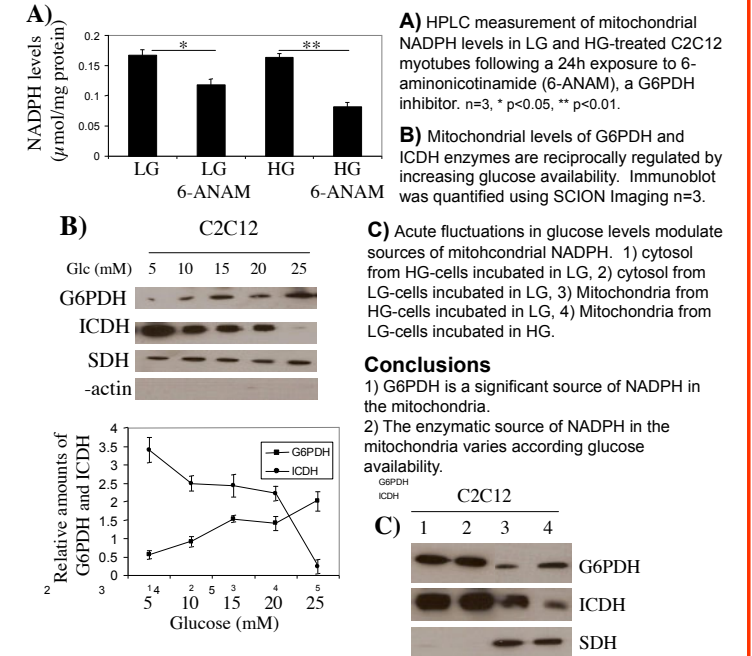


Conclusions

- 1) The activities of mitochondrial G6PDH and ICDH are reciprocally regulated by glucose availability.
- 2) G6PDH is associated with the matrix.
- 3) Similar results were obtained from HEK293 cells

Results

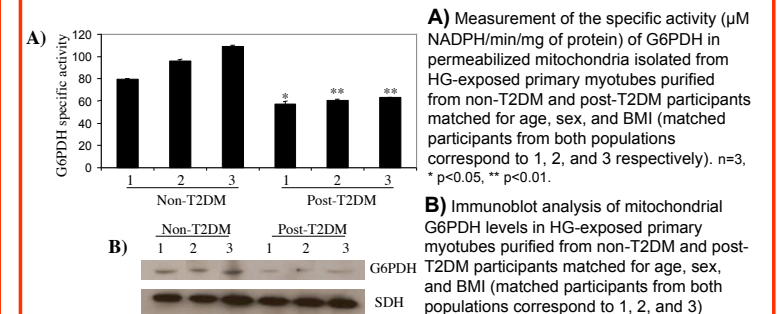
Figure 3: G6PDH and ICDH are regulated by glucose availability



Conclusions

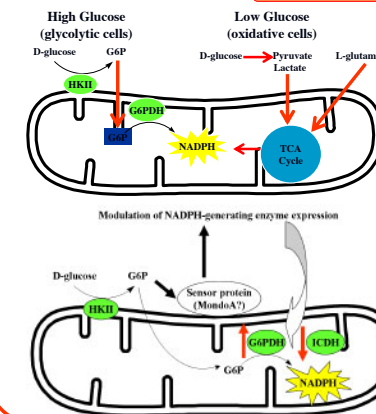
- 1) G6PDH is a significant source of NADPH in the mitochondria.
- 2) The enzymatic source of NADPH in the mitochondria varies according to glucose availability.

Figure 4: Mitochondrial G6PDH in post-T2DM



Conclusion: 1) Primary myotubes from post-T2DM participants are unable to increase mitochondrial G6PDH which perpetuates oxidative stress and mitochondrial ROS formation 2) Despite improved aerobic metabolism, primary myotubes from post-T2DM participants are unable to properly respond to increased glucose levels. 3) These data indicate glucose sensing plays a part in modulating the source of mitochondrial NADPH.

Summary



- 1) G6PDH is associated with the matrix of mitochondria
- 2) G6PDH plays a key role in providing mitochondrial NADPH
- 3) Glucose availability regulates the mitochondrial enzymatic source of NADPH.
- 4) Nutrient sensing plays a role in dictating the enzymatic source of NADPH in skeletal muscle mitochondria
- 5) Perturbed glucose sensing in post-T2DM skeletal muscle leads to the decreased capacity to produce NADPH under high glucose conditions.

Acknowledgements

