Investigating oxidative stress in weight loss variability- a preliminary study

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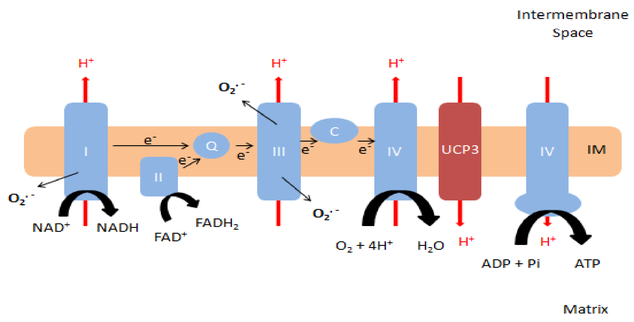
Introduction

Reactive oxygen species (ROS) production in mitochondria is known to cause extensive cellular damage, known as oxidative stress. Small increases in ROS production activates mitochondrial proton leak through an uncoupling protein, UCP3. UCP3 allows H+ to move across the mitochondrial inner membrane independent of ATP production, reducing the electrochemical gradient across the membrane and alleviating oxidative stress (Mailloux and Harper, 2011). Previous studies in the Harper laboratory have determined that individuals in the highest quintile for rate of weight loss (Obese Diet Sensitive; ODS) have higher rates of mitochondrial proton leak than do individuals in the lowest quintile for rate of weight loss (Obese Diet Resistant; ODR) and thus may be protected against oxidative stress.

PURPOSE: The current study was designed to investigate the effects of oxidative stress on mitochondrial respiration in myotubes derived from ODS and ODR individuals. Prior to initiating studies investigating oxidative stress, preliminary studies were conducted to assess appropriate seeding density and differentiation media concentrations.

HYPOTHESIS: It is hypothesized that myotubes derived from ODS individuals will have higher rates of mitochondrial proton leak in response to oxidative stress treatment compared to ODR.

Biological Background



The Electron Transport Chain (ETC), located on the mitochondrial inner membrane, is the largest source of cellular ATP. Production is driven by a proton gradient established by the ETC complexes. The ETC accepts electrons from reduced metabolic substrates and shuttles them through a series of oxidation- reduction reactions to the final electron acceptor O_2:

$$2\ e^- + 2\ H^+ + \tfrac{1}{2}\ O_2 \rightarrow H_2O$$

Single electrons can be released from some ETC complexes, causing an incomplete reduction of oxygen to form ROS (Mailloux and Harper, 2012).

Results

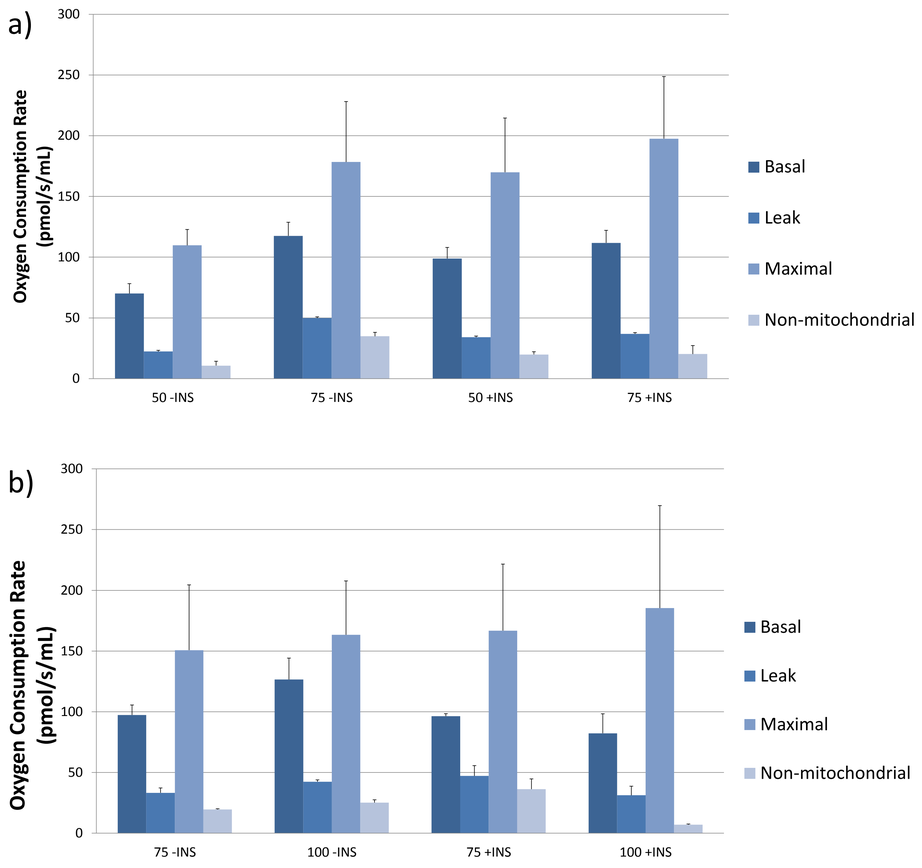


Figure 1: Effects of cell density and the presence of insulin in differentiation media on the respiration rates of human myotubes.

Figure 1: Effects of cell density and the presence of insulin in differentiation media on the respiration rates of human myotubes. a) Cells were plated at a density of 50,000 or 75,000 cells and differentiated +/- insulin. N=3, 5 replicates per condition per experiment. B) Cells were plated at a density of 75,000 or 100,000 cells and differentiated +/- insulin. N=2, 5 replicates per condition per experiment . Data are mean +/- SEM. Oxygen consumption rates were measured at four respiration states using a Seahorse Bioscience XF-24 analyzer. Basal respiration rates were produced by regular mitochondrial functioning. Leak-dependent respiration was induced by inhibiting ATP synthase(oligomycin; 600ng/mL). Maximal respiration was induced by the chemical uncoupler Carbonyl cyanide-p-trifluoromethoxyphyenylhydrazone (1uM) to completely uncouple oxygen consumption from ATP production. Non-mitochondrial respiration was induced by the inhibition of complex III in the electron transport chain (antimycin A; 4uM).

Conclusions

- It has been established that a density of 100,000 cells is optimal for measuring respiration in human myotubes.
- The presence of insulin in the differentiation media produces a more accurate Maximal: Basal respiration ratio.

Future Work

The next step in this project is to determine the effects of oxidative stress induced by 24 hr treatment with 500uM palmitate treatment or 1 hr treatment with 200uM hydrogen peroxide) on mitochondrial respiration in ODS and ODR myotubes.

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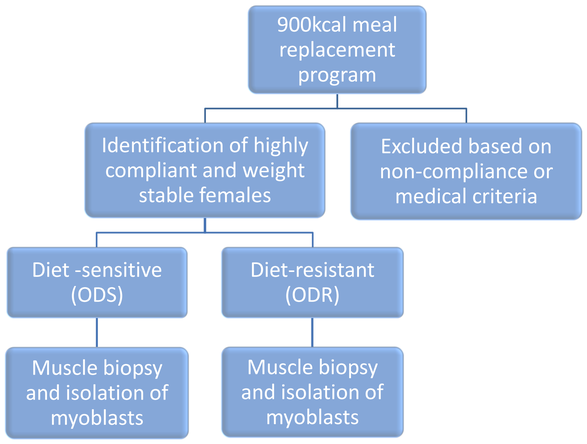
References

Mailloux, R. and Harper, ME. (2012). Mitochondrial proticity and ROS signaling: lessons from the uncoupling proteins. *Trends in Endocrinol Metab*. Vol. 23(9)

Mailloux, R and Harper ME. (2011). Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radical Biology and Medicine*, Vol. 51(6)

Harper, ME et. al. (2002) Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. *Diabetes*, Vol. 51

Methods



Myoblasts were previously isolated from the *vastus lateralis* muscle of highly compliant ODR and ODS individuals following the completion of a 900 kcal meal replacement program. Participants were matched for age and initial weight (Harper, ME. et. al. 2002).

Before experiments could be performed to assess cellular response to ROS production, preliminary experiments were conducted to determine the optimal conditions, including appropriate cell density, and whether insulin should be added to or excluded from the differentiation media.

The isolated myoblasts were grown to confluence on matrigel-coated flasks in F10 media (12% Fetal Bovine serum, 1% antibiotic-antimycotic, 2.5ug/mL gentamycin, 1umol/L dexamethazone, 10ng/mL epidermal growth factor). Myoblasts were subsequently added to coated microplates at densities of 50 000, 75 000, or 100 000 cells. Cells were changed to differentiation media (low glucose Dulbecco's Modified Eagle's Medium DMEM, containing 5mM glucose, 4mM glutamine, 1mM pyruvate, 2% horse serum, 1% antibiotic-antimycotic and2.5ug/mL gentamycin) the following day. All experiments were conducted following 7 days of differentiation. To determine the effects of insulin on cellular differentiation and mitochondrial respiration, cells were differentiated +/- 25pM insulin. Oxygen consumption of the mature myotubes was measured using a Seahorse Bioscience XF-24 analyzer.