Project Description

Title: DGAT1/2 Knockout Cell Characterization
Student: Nicholas Brownell
Advisors: Dr. Charlie Harris and Dr. Bob Farese, Dr. Dan Radar

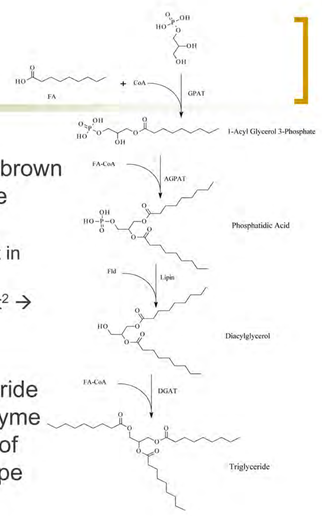
Scientists know that the enzyme DGAT is involved in the formation of cells that store fat in the body and that the ability to control fat-cell formation might help clinicians fight the current obesity epidemic in America. Nicholas studied mouse fat cells that had DGAT removed, hoping its removal would prevent fat storage cell formation. While he found that removing DGAT did not fully prevent the formation of fat cells, he was able to gain insight into how DGAT affected these fat cells.

BE-490 Fall 2009: DGAT1/2 Knockout Cell Characterization

Nicholas K. Brownell

Dr. Charlie Harris and Dr. Bob Farese, Gladstone Institute of Cardiovascular Disease
Dr. Dan Rader, Department of Medicine, University of Pennsylvania

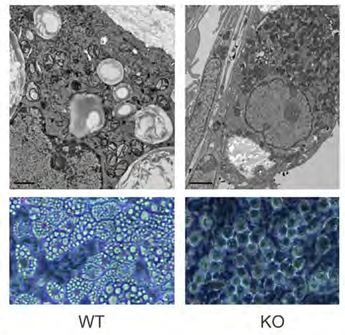
Purpose



- Is it possible to create a brown adipocyte from adipocyte precursors?
  - White adipocytes store fat in triglycerides[2] → obesity
  - Brown adipocytes burn fat[2] → solution to obesity?
- Want to alter the triglyceride pathway through an enzyme knockout (KO) in hopes of forcing a brown phenotype

Background

- DGAT: Catalyzes the final step of triglyceride formation[3]
- Previous KO studies *in vivo*
  - DGAT1: Protected from obesity[1]
  - DGAT2: Die shortly after birth[1]
- Previous KO studies *in vitro*
  - KO cells have more mitochondria than WT cells
  - Brown adipocytes have more mitochondria than white adipocytes
- Is a DGAT1/2 KO cell actually a brown adipocyte?

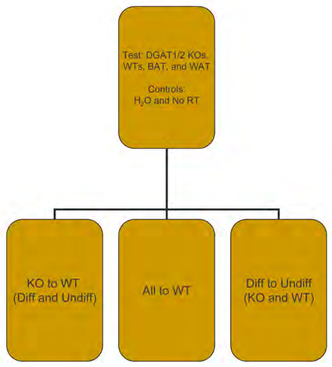


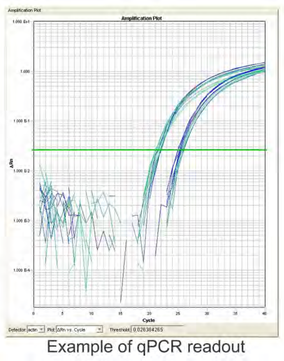
WT KO

Hypotheses

- The DGAT1/2 KO cell will resemble an adipocyte
  - Adipocyte genes – expected to have higher expression levels in the differentiated cell than the undifferentiated cell, as verified by qPCR
- The DGAT1/2 KO cell will resemble a brown adipocyte in terms of gene expression, as compared to the WT and verified by qPCR
  - Brown adipocyte-specific genes – expected to be higher in KO
  - White adipocyte-specific genes – expected to be lower in KO
  - Mitochondrial genes – expected to be far higher in KO
- Characterize the DGAT1/2 KO cell
  - Other genes for overall characterization (hormones, transcription factors, triglyceride formation genes, etc.)
- Compare gene amplifications in KO and WT to brown adipocytes (BAT) and white adipocytes (WAT)

Diagram and Project Illustration

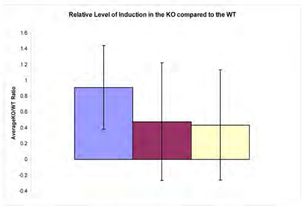




Example of qPCR readout

Results

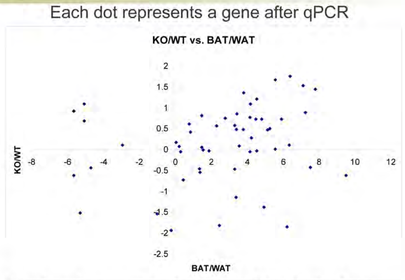
- 36 of 55 genes total had higher expression in the Diff. compared to Undiff. (KO condition, $p<0.05$)
- Higher expression in KO, compared to WT (Diff. condition, $p<0.05$)
  - 7 of 13 brown adipose genes (1 lower)
  - 2 of 5 white adipose genes (1 lower)
  - 3 of 7 mitochondrial genes
- 0.4 to 0.9 fold induction (avg) in KO compared to WT, regardless of gene association (graph, right)



Blue – Mitochondrial brown adipose genes
Purple – Nonmitochondrial brown adipose genes
Yellow – White adipose genes

Results

Each dot represents a gene after qPCR



KO induction is above the X-axis; WT is below
Brown genes are to the right of the Y-axis; white on the left

Majority of genes (both brown and white) are KO induced

Conclusions and Acknowledgements

- KO cell has an adipocyte gene expression profile
  - Diff. induced most genes tested, as compared to Undiff.
- KO cell is a "mixed" adipocyte – part white, part brown
  - White adipocyte and brown adipocyte genes induced equally
  - Mitochondrial gene induction was not significantly different than any other genes
  - Overall characterization confirms adipocyte expression profile
- Many thanks to Dr. Charlie Harris, my mentor throughout the project
- Thanks to Dr. Bob Farese and Dr. Dan Rader for overseeing the project, both at UCSF and at UPenn

References

1. Chen, H.C., Stone, S.J., Zhou, P., Buhman, K.K., & Farese, Jr., R.V. (2002). Dissociation of obesity and impaired glucose disposal in mice overexpressing acyl coenzyme A:diacylgylcerol acyltransferase 1 in white adipose tissue. *Diabetes*, 51(11), 3189-95.
2. Nedergaard, J., Bengtsson, T., & Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology - Endocrinology and Metabolism*, 293, 444-52.
3. Takeuchi , K., & Reue, K. (2009). Biochemistry, physiology, and genetics of gpat, agpat, and lipin enzymes in triglyceride synthesis. *American Journal of Physiology - Endocrinology and Metabolism*, 296(1), 195-209.