How Does Inflammation in the Adipose Tissue Contribute to Diabetes?

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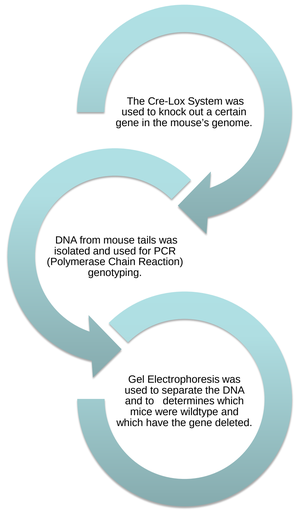
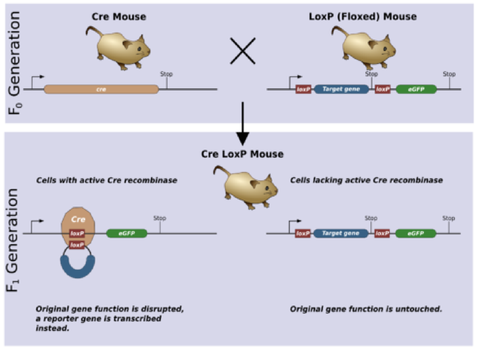
Introduction: Type 2 Diabetes is a metabolic disorder due to the development of insulin resistance in cells throughout the body. We are studying how inflammation in adipose tissue contributes to insulin resistance and the onset of diabetes. Excess lipids in the cells of adipose tissue (lipid overload) cause the cells to leak the excess fatty acids leading to higher DAG (diacylglycerols) levels, a type of secondary messenger to accumulate in muscle cells. The accumulation of DAG interferes with the insulin receptor signaling and inhibits insulin from regulating glucose levels[1]. We use conditional mouse models to knock out certain inflammatory signaling molecules in the adipose tissue and investigate how this affects insulin resistance in adipose tissue and other insulin sensitive organs. To study the adipose tissue of the mice we have to collect cell cultures which we turned into histology sections. We looked at histology sections of fat tissue and assessed the number of fat cells and the number of apoptotic cells in wild type mice versus knockout mice to see if there is a difference in the number of cells.

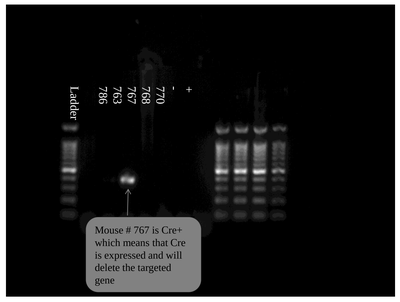
Conditional Mouse Models to Delete a Gene in Adipose Tissue

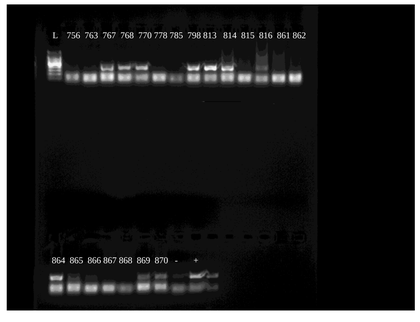


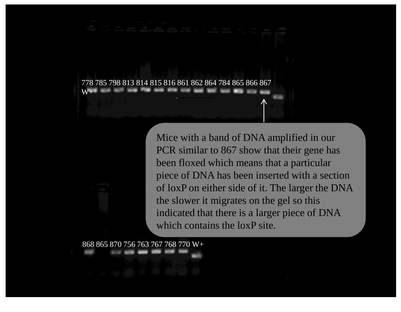


Schematic Diagram of Cre-Lox System[2].

Cre is a DNA recombinase protein which catalyzes the recombination of DNA between specific target sites. LoxP is a sequence of DNA that flanks two sides of a DNA section which you would recombine with Cre. LoxP are the specific target sites. The Cre is able to only affect the adipose tissue in a mouse because we place it after a certain promoter (AP_2) in the DNA that is specific to the cells in adipose tissue.







References:

1.Taubes, G (2009) Prosperity's Plague. SCIENCE 325, 256-260.
2.http://en.wikipedia.org/wiki/Cre-Lox_recombination

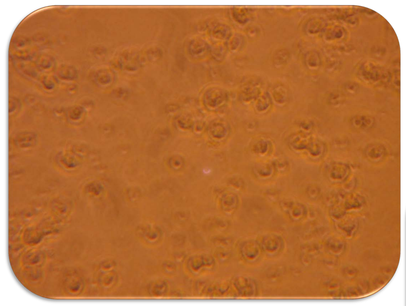
Cell Culture of Peritoneal Macrophages

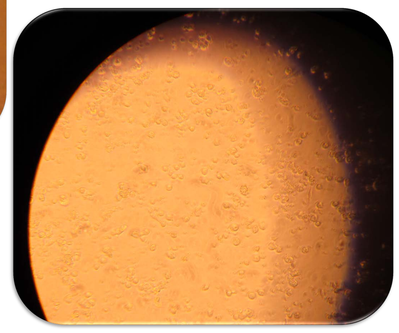
1
- We injected 3% thioglycolate into the peritoneal cavity of the mice which irritates the mice cells and attracts macrophages.

2
- Two days later, the macrophages that accumulated because of the injection were flushed out of the peritoneal cavity.

3
- The collected cells were then washed and the red blood cells were lysed.

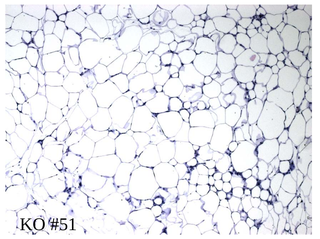
4
- The cells were counted and plated at a density of 2.5 X 10^5 cells per well in a 24 well plate. Medium DEMEM 10% FCS P/S

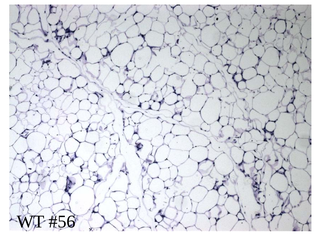


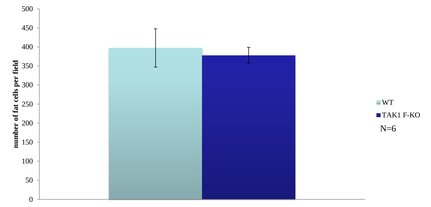


Here are some pictures of the cells I cultured. Unfortunately after three days the wells without antibiotics were infected with bacteria.

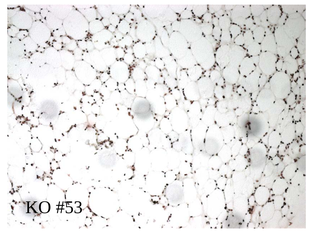
Histology

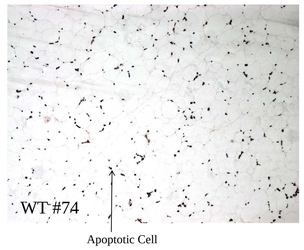


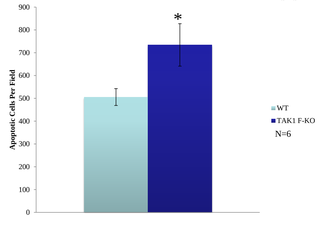




There was not much difference between the cell counts of the wildtype and knockout mice. This means The obese knockout mice have the same amount of cells compared to obese wild type mice.







We used TUNNEL assay to label the dead, apoptotic cells. There was more apoptosis in the fat tissue of obese knockout mice compared to the obese wild type mice.

Conclusion: We can conclude that the knockout gene affects cell viability.