Changes in glutathione to glutathione disulfide ratio after insulin treatment in primary mouse myoblast cells

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Abstract

Background: Glutathione (GSH) is the major endogenous antioxidant in cells. Differences in its redox state are indicative of redox status within the cell and consequently provide an indication of oxidative stress. In response to higher levels of reactive oxygen species (ROS), GSH is oxidized to glutathione disulfide (GSSG). Reduced GSH is a linear tripeptide of L-glutamine, L-cysteine, and glycine. The molecule has a sulfhydryl (SH) group on the cysteinyl portion, which accounts for its strong electron-donating character. As electrons are lost, the molecule becomes oxidized, and two such molecules become linked (dimerized) by a disulfide bridge to form GSSG. This linkage is reversible upon reduction.

Introduction

Insulin is a peptide hormone produced by beta cells in the pancreas. It is responsible for regulating blood glucose levels and as a result it regulates the metabolism of carbohydrates. This is done by regulating the absorption of glucose into skeletal muscle and adipose tissue.

GSH is one of the antioxidants found in primary mouse myoblasts. When GSH is oxidized it dimerizes to form GSSG through a disulphide bond. Antioxidants are responsible for preventing damage caused by reactive oxygen species.

The purpose of this experiment was to assess wether insulin treated primary myoblasts affect the GSH:GSSG ratio.

Reactive Oxygen Species (ROS)

ROS are oxygen molecules with a free radical, they can cause damage to the cell in high quantities by oxidizing intracellular macromolecules such as DNA, protein and lipids. In lower quantities they are essential for cellular signalling pathways. They are also known to cause aging. The role on intracellular antioxidants is to negate the harmful effects of ROS preventing cellular damage.

Much of the ROS production is via the electron transport chain within the mitochondrial matrix, when oxygen accepts a single electron from one of the protein complexes that make up the chain.

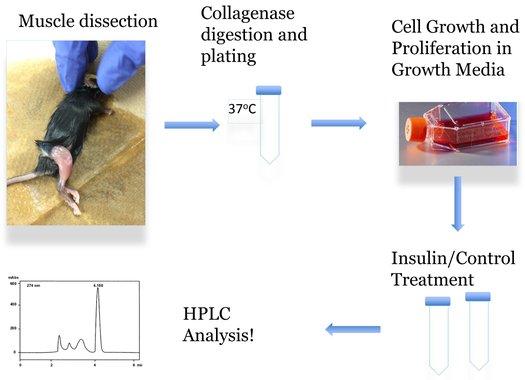
ROS can be visualized using fluorescent microscopy. Cells are transacted with Lipofectamine 2000 transfection agent with a DNA sequence coding for green fluorescent protein. The green fluorescent protein responds to GSH/GSSG redox and can be visualized under the microscope.

Hypothesis

Preliminary results indicate that insulin treatment affects GSH/GSSG ratio. We hypothesize that the insulin treated cells will have a different GSH/GSSG ratio then the control group.

Insulin Is a key peptide hormone stimulating glucose uptake into primary mouse myoblasts. Glucose is a carbohydrate that can be oxidized resulting in ATP production. Glucose metabolism begins with glycolysis which produces pyruvate, pyruvate enters the mitochondria and undergoes the link reaction with Acetyl CoA, followed by the TCA Cycle.

Methods



High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is an analytical chemistry technique that can be used to separate and identify relative quantities of particular molecules within a mixture. The lysate from the cells are run through the HPLC machine.

The HPLC machine uses high pressure to move the lysate and mobile phase through a stationary phase. As the lysate passes through the stationary phase, the contents are then separated and peaks are measured by the computer.

Results

Results so far suggest a correlation between insulin treatment and GSH:GSSG ratio in primary mouse myoblasts. Further more tests are needed to confirm results and understand different processes that may be at work.

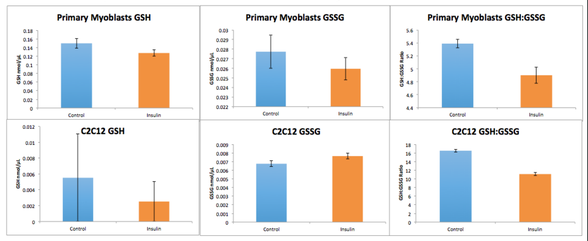
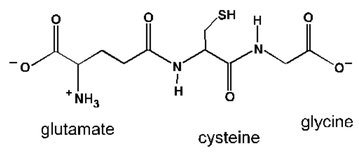


Figure 1 – Results from HPLC tests measuring GSH:GSSG ratio in primary mouse myoblasts. Samples were taken from 3 separate plates, combined and lysed. Insulin treated and control cells were diluted and separated into two sets, the first 20ul and the second 40ul. The values were obtained using the area under the curve produced by the HPLC machine. The peaks were identified using standard for GSH and GSSH which were run through the machine prior to injecting the samples. The viability of the cells was measured in order to control for varying levels.

glutathione (GSH)



Discussion

We hope that this ongoing research investigating the effect of insulin treatment on GSH:GSSH levels will conform the correlation between insulin levels and GSH:GSSH ratio in primary mouse myoblasts. The results show a difference between the insulin treated and and control samples.

In primary myoblasts the control shows higher levels of both GSH and GSSG and the ratio of GSH:GSSG is higher.

Discussion Continued…

This is an interesting result as it was expected that is GSH levels in control cells were higher than GSSG then the GSSG levels would be lower, this implies that GSH may be have been used elsewhere in the cell. More tests are needed to identify possible causes of the difference between observed and expected results.

Primary myoblasts show a higher GSH:GSSG ratio the in the control then insulin treated cells implying there is more GSH relative to GSSG in the control group then the insulin treated group. The opposite is true in the C2C12 cancer cell line.

Results From Microscopy

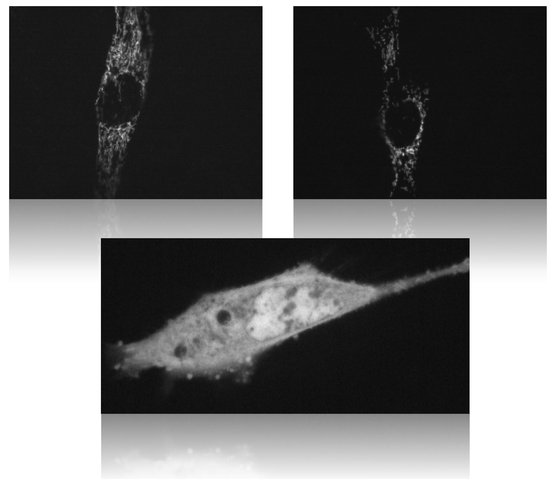
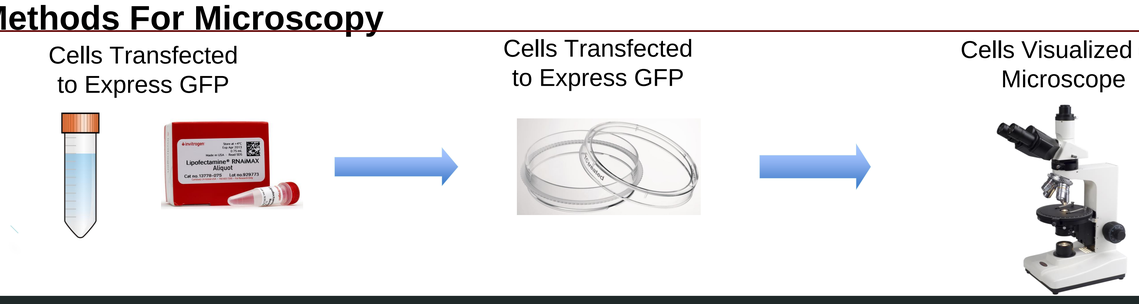


Figure 2 – Cells transfected with Green Fluorescent Protein (GFP) using Lipofectamine 2000. Visualized at 63x magnification. GFP is sensitive to redox ratio in the cell through interaction with glutathione. The first two pictures show GFP expression in mitochondria whereas the last picture shows cytoplasmic GFP expression. Results from these tests reinforce the results obtained from HPLC analysis. Bright areas show more interaction with glutathione and dark areas show less. These images were taken of the c2c12 cancer cell line.

Discussion From Microscopy

The images show significant GFP expression in the c2c12 cell line. Because the GFP is sensitive to redox ratio expression in the form of green flashes indicate, changes in glutathione redox ratio.We will continue transfecting cells with GFP in order to visualize oxidization in the primary mouse myoblasts and c2c12 cell line to provide further evidence of results. More tests will be done to conform our findings.

Methods For Microscopy



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