

The Effects of Varying Glucose Concentrations on Cellular Glycolytic Rates

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BRIEF. A research paper on defining the redox ratio, a biomarker of cancer therapy effectiveness.

ABSTRACT. Cells use several cellular respiration pathways including glycolysis and oxidative phosphorylation, to convert glucose into carbon dioxide, water, and ATP for the cell to use as energy. The optical redox ratio is a relative measure of the rates of glycolysis versus oxidative phosphorylation, and is poorly characterized in regards to sensitivity to metabolic inputs. Our objective was to determine the sensitivity of the redox ratio to cellular glucose concentrations. We expected rates of glycolysis to increase with media-glucose concentration, resulting in an increased redox ratio. At increased media-glucose concentrations, we expected higher rates of glycolysis resulting in increased cellular concentrations of NADH and decreased FAD concentrations. For this study, MCF7 cells were grown *in vitro* with media supplemented with glucose at concentrations between 0 mM and 22.7 mM. Cellular NADH and FAD fluorescence was imaged by multi-photon fluorescence microscopy. The media containing no glucose, 0 mM, had the greatest effect on the glycolytic rates of cancer cells (p-value = .01).

INTRODUCTION.

Our bodies are composed of trillions of cells that all play different parts with a diversity of functionalities. All cells use cellular respiration to make ATP (adenosine triphosphate) for the cells to use as energy. Mutations in DNA during replication, when cells divide into newer cells, can result in cancer cells. Approximately 30% of all cancer cases among American women are breast cancer [1]. Advances in health care and technology have allowed for extensive research into understanding this deadly disease. Current clinical therapies include chemotherapy, a process in which the chemotherapy drug attacks any cells that are rapidly dividing. Unfortunately, these non-specific drugs cannot distinguish between cancerous cells and healthy cells [2].

Cancer cells *in vitro* are used to test novel drug and treatment. Two-photon fluorescence microscopy utilizes a high power laser to excite tissue fluorescence. Researchers are looking for better treatments, or possible cures, for various types of cancer, and, by acquiring a baseline redox ratio, scientists will have a normal to compare all data involving cancer and the redox ratio to, which can help with the understanding of the effectiveness of various treatments against cancer. Fluorescence intensity is proportional to fluorophore concentration and can thus probe relative concentrations of biological molecules. Two endogenous fluorophores, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), are coenzymes of metabolism and report cellular metabolism health and efficiency. The redox ratio measures the glycolytic rates of the cells, as NADH is created in glycolysis, and FAD is used in oxidative phosphorylation [3]. The redox ratio is sensitive to metabolic changes induced by malignancy and cancer treatments [4] however, the sensitivity of the redox ratio to changes in available glucose has not been defined. The following experiments attempt to define the relationship between the redox ratio and cellular glucose concentrations. This knowledge is significant for the future of cancer research, because the results will further define the redox ratio, which is under development as a biomarker of cancer therapy effectiveness.

MATERIALS AND METHODS.

Materials.

We used the MCF-7 cell line, which is estrogen receptor positive (ER+) [5]. To culture the MCF-7 cells, cell growth media (DMEM) was used with varying amounts of glucose (and glutamine), such as 0 mM (0 mM), 2.5 mM (.396 mM), 5 mM (.792 mM), 7.5 mM (1.188 mM), 10 mM (1.584 mM), 15 mM (2.376 mM), 22.7 mM (3.6 mM). The growth media was supplemented with

10% fetal bovine serum (FBS) and, 1% penicillin: streptomycin, an antibiotic/antifungal. Cells were cultured in an incubator, which maintained the temperature at 37°C, the humidity at 95%, and the CO₂ at 5%.

Optical metabolic imaging interrogates the intrinsic fluorescence of NADH and FAD. Two-photon microscopy was performed on a customized multi-photon fluorescence microscope (Prairie Technologies) utilizing a titanium-sapphire laser (Chameleon, Coherent) tuned to 750 nm for NADH fluorescence and tuned to 890 nm for FAD fluorescence, as the excitation source. Two objectives, 40x and 20x (NA of 1.15, and 0.75, respectively), coupled the excitation and emission light in the epi-configuration. Two filters isolated 400-480 nm NADH emission and 500-600 nm FAD emission.

Time Course Methods.

A time course was used to determine how length of time required for the MCF-7 cells to adjust their glycolytic rates to the new glucose concentration. The cells were imaged after exposure to 0, 5, and 22.7 mM glucose for 0, 3, 6, and 24 hours. At each time interval, five different fields of view on each dish were imaged for repeated measurements. The images were analyzed by applying an intensity threshold to isolate cytoplasm fluorescence in ImageJ, a computer software image analyzer. Redox ratio values were obtained by dividing the NADH image by the FAD image to acquire a redox ratio value for each pixel. Cellular redox ratio values were computed by averaging the cytoplasm pixels for each cell. Cell mean redox ratio values were averaged across the image.

Final Methods.

To investigate additional glucose media concentrations, MCF7 cells were plated and placed in 22.7 mM glucose media (normal, high glucose DMEM), for 24 hours. After 24 hours, the media was exchanged with the media of the following concentrations of glucose: 0 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 15 mM, 22.7 mM. The cells were grown for another 24 hours to acclimate to the new media and its level of glucose. After that 24 hour time period, the cells were imaged and the images analyzed in the exact same way as the time course methods. The only difference was the use of a 20x objective (NA of 0.75) instead of the 40x objective.

RESULTS.

Time Course Results.

The time course data was normalized to the 22.7 mM solution, since that concentration of glucose was the standard high glucose media. The redox ratio was expected to decrease in cells exposed to the 0 mM concentration glucose media, however we observed no significant change in the redox ratio of the 0 mM and 5 mM after exposure for 24 hours (Figure 1). To validate metabolic interpretation from the redox ratio, the NADH and FAD fluorescence counts were examined separately. No change in NADH fluorescence was observed between the low-glucose media concentrations at any time point (Figure 2).

Final Methods Results.

To determine the effect of the low metabolite concentrations on the redox ratio, both the glucose concentration and glutamine concentrations were varied. The redox ratios from cells with the glucose concentrations between 5 and 22.7 are statistically similar, with the exception of the 10 mM solutions. The cells grown in 0 mM glucose had a statistically significant decrease in the redox ratio (Figure 3). Without glucose, cells cease producing ATP through glycolysis and glucose-driven oxidative phosphorylation. Without glucose, the cells can use stored metabolites such as amino acids for metabolism, which may result in a decrease in the redox ratio.

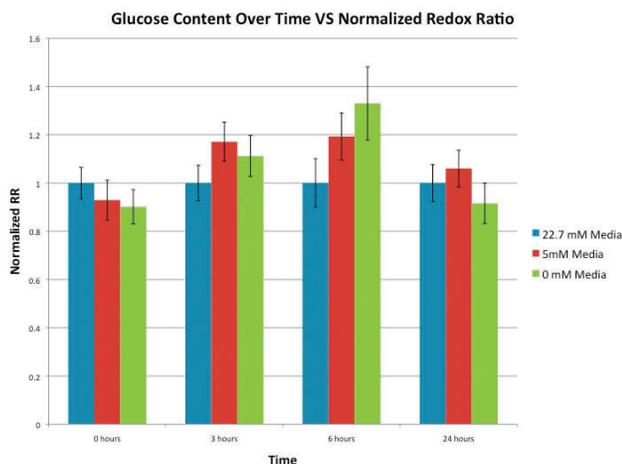


Figure 1. Time Course Redox Ratios for 22.7 mM, 5 mM, and 0 mM Media. All data is normalized to the 22.7 Media RR.

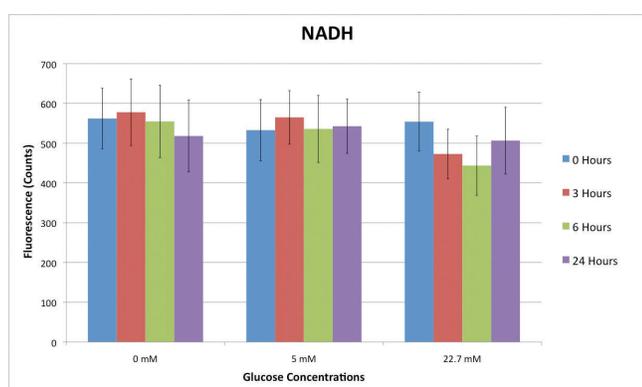


Figure 2. NADH Fluorescence counts from the Time Course Data.

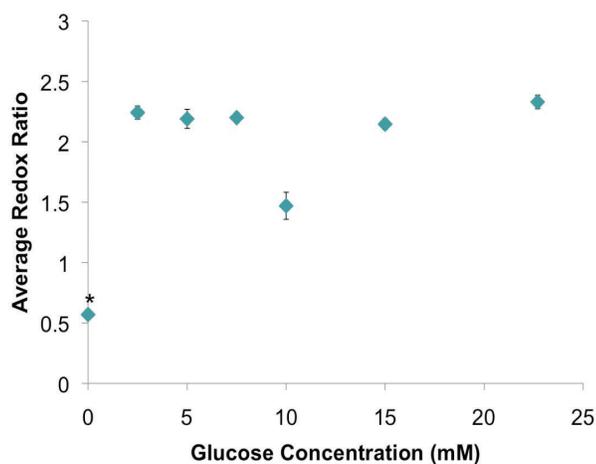


Figure 3. Scatter Plot of data from the Final Methods. The asterisk next to the 0 mM means that it is statistically significant using a student's T-test with a P-value of .01.

DISCUSSION.

Within the time course, the lack of change in redox ratio with decreased media glucose could be due to a metabolic stress effect from changing media or the presence of additional metabolites, such as glutamine, in the media. Glutamine can enter the oxidative phosphorylation pathway to produce ATP, which would increase FAD and reduce NADH concentrations [6]. Increased glutamine metabolism may result in an increase in the redox ratio, which was observed in the low glucose, high glutamine media (Figure 1). For the FAD fluorescence, while not significant due to large error, a trend towards increased in FAD fluorescence was observed in the cells exposed to the low glucose concentration media at longer times, 6 and 24 hours (Figure S1). An increase in FAD fluorescence would suggest an increase in oxidative phosphorylation, consistent with increased glutamine metabolism.

While in the final method, the similar redox ratios among cells grown in media of glucose concentrations between 5 and 22.7 mM, could be attributed to either equal reductions in glycolysis and oxidative phosphorylation rates or a low-glucose saturation level.

Overall, increased media-glucose concentrations, above 2 mM, appeared to have no effect on cellular metabolism function. Future research efforts include experiments to test glucose concentrations between 0 mM to 2.5 mM and to allow an increased acclimation time in order to look at possible differences.

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