Determining Vitamin C's effect on the proliferation and viability of endothelial cells in a high glucose environment

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Introduction

High glucose-induced apoptosis

Excess glucose metabolized in glycolysis & citric acid cycle

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Excess NADH & $FADH_2$

↓

Excess electrons for donation to ETC

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High mitochondrial membrane potential

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ETC inhibition

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Oxygen reduced to form an excess of superoxide radical

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Induction of programmed cell death

(Brownlee, 2001; Hou et al., 2015)

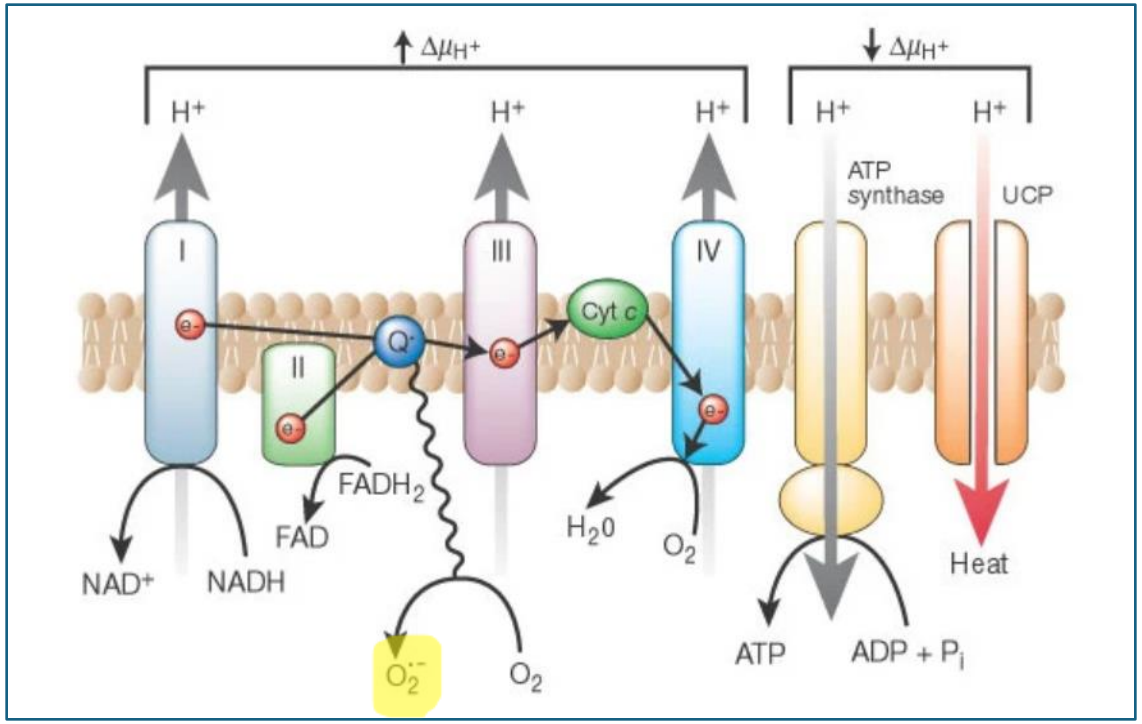

Figure 1. The electron transport chain and it's relation to mitochondrial membrane potential and ROS production *(Brownlee, 2001)*.

Introduction

The potential of Vitamin C

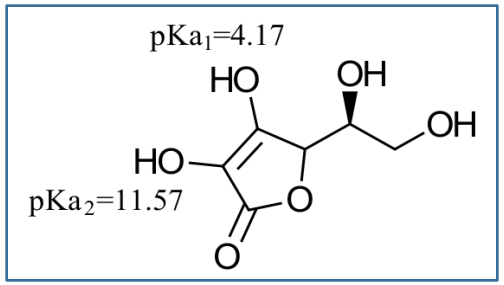

Figure 3. Ascorbic acid structure and pKa values from PubChem (*drawn in ChemDoodle*).

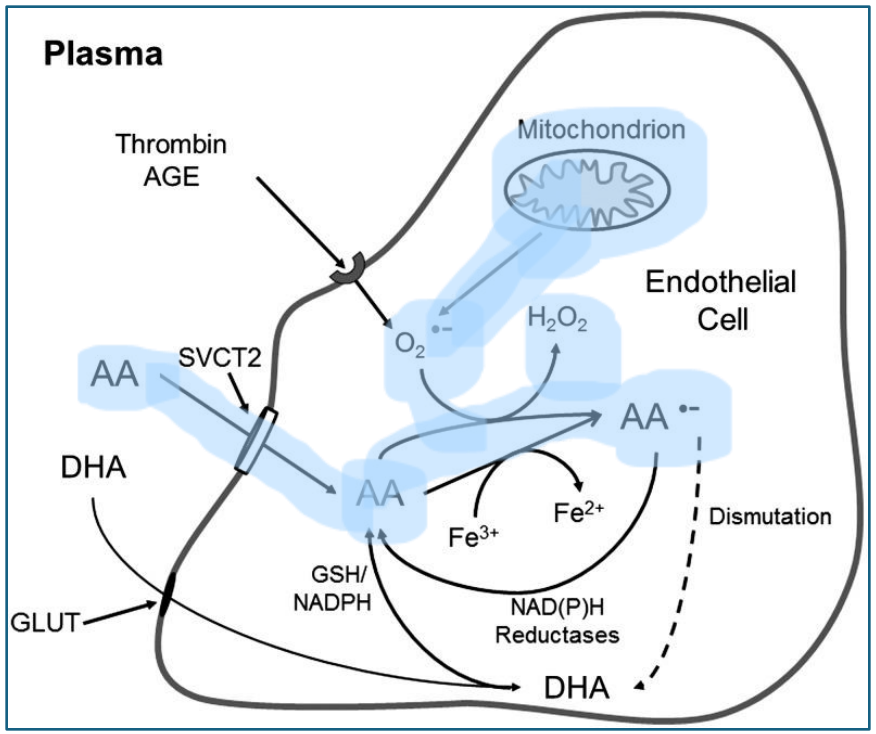

Figure 2. Ascorbate (AA) reactions in endothelial cells. Oxidation of superoxide radical to hydrogen peroxide using AA highlighted *(May & Harrison, 2013)*.

- Important to endothelial cell health
- Acts as an antioxidant (Figure 2)
- Potential to prevent cell death caused by high glucose

  (May & Harrison, 2013)
- Previous study showed vitamin C inhibited high glucose-induced apoptosis in endothelial cells
- Hyperglycemia:
  - High blood-glucose levels
  - Significant in diabetes

  (Feng, et al., 2000)

Our Hypothesis

If vitamin C is supplemented to endothelial cells exposed to high glucose levels, a reduction of cell death will be observed using hemocytometry and the bicinchoninic acid assay.

Methodology

Cell Cultures & Hemocytometry

- 3 culture media
  - Control (Leibovitz's L-15, 10% fetal growth serum,, antibiotic/antimycotic)
  - Control + D-Glucose (30 mM)
  - Control + D-Glucose (30 mM) + Vitamin C (300 µM)

- 3 flasks of Ea.hy926 endothelial cells
  - Control
  - Just glucose
  - Glucose + Vitamin C

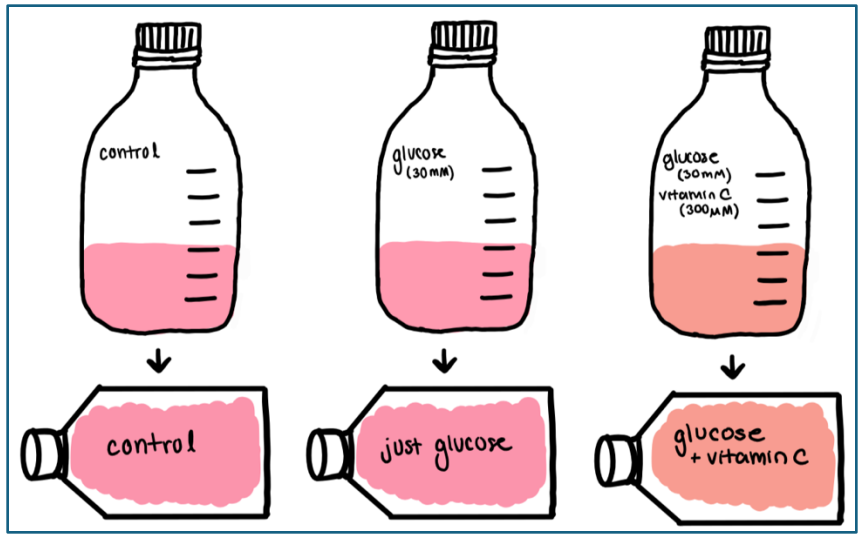

Figure 4. Diagram of experimental media and cell cultures.

- Flasks counted via hemocytometry & microscopy at ohrs, 24hrs, & 48hrs
  - Procedure for cell suspension in liquid from Biol3520 lab manual followed

- Cells incubated at 37° in 5% CO_2

Methodology

Bicinchoninic acid assay

- Prepared BSA standards (3 replicates) with concentrations of 0, 0.10, 0.25, 0.50, 0.75, & 1.00 mg/mL
- After counting at 48hrs, lysed each cell culture
  - Followed Biol3520 lab manual procedure for animal cell lysis & BCA
- Prepared 3 replicate samples for control, just glucose, & glucose + vitamin C cells
- Using the NanoDrop:
  - Measured standards to form standard curve
  - Measured protein content of experimental samples
- ANOVA to determine significance of results

Results

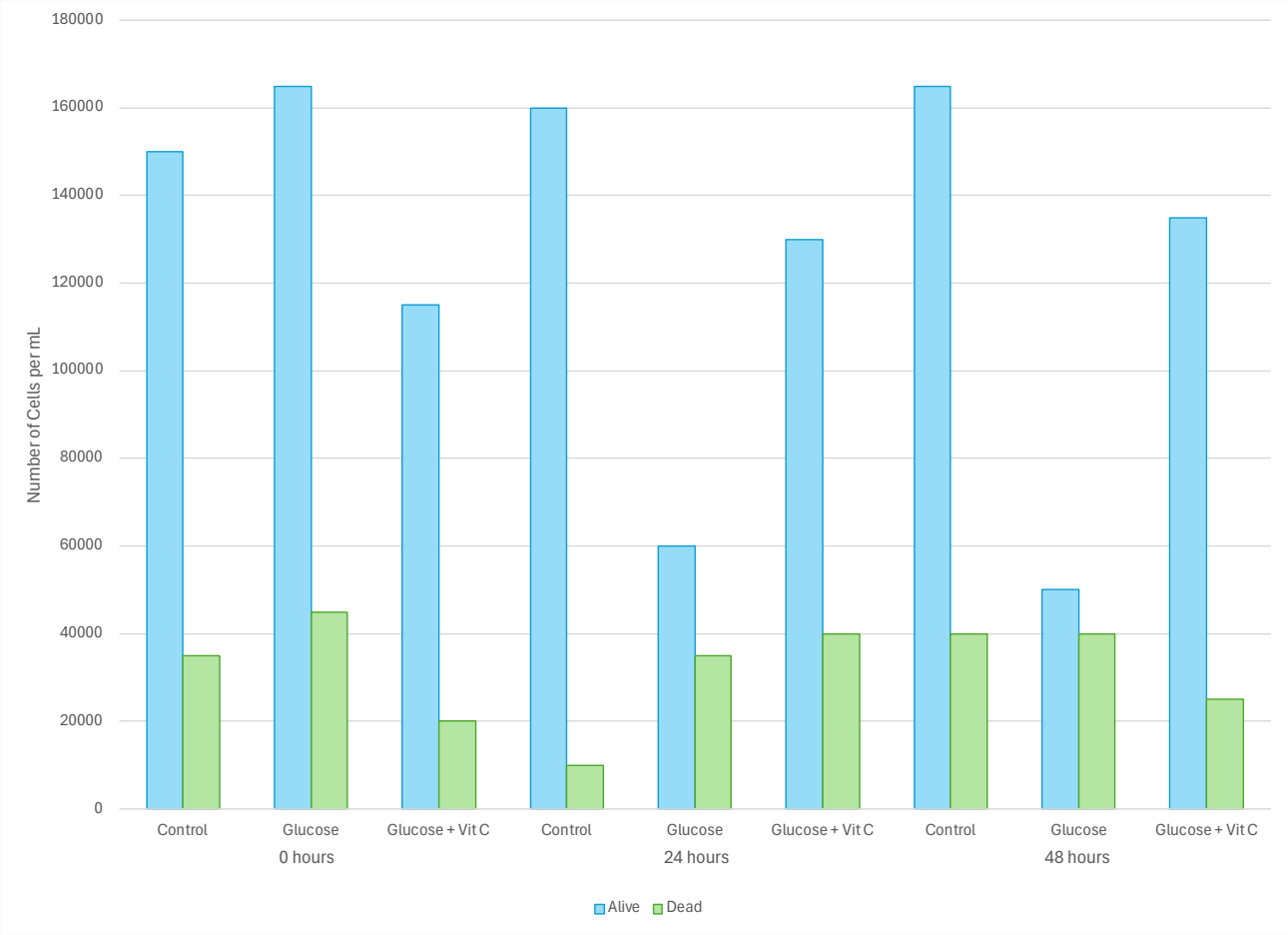

Figure 5. Count of alive and dead cells for control, glucose, and glucose + vitamin C flasks at 0, 24, and 48 hours.

- Control Treatment
  - Number of alive cells relatively stable over 48 hours.
  - Dead cell count peaked at 48 hours but also remained relatively stable over time.
- Glucose Treatment
  - Significant decrease of alive cells from 0 to 24 hours.
  - Alive cell count was over two times fewer at 48 hours than at 0 hours, as well as both other treatments at 48 hours.
- Glucose + Vitamin C Treatment
  - Maintained a high number of alive cells across all time points.
  - Increase in the number of dead cells after 24 hours, which decreased after 48 hours.

Results

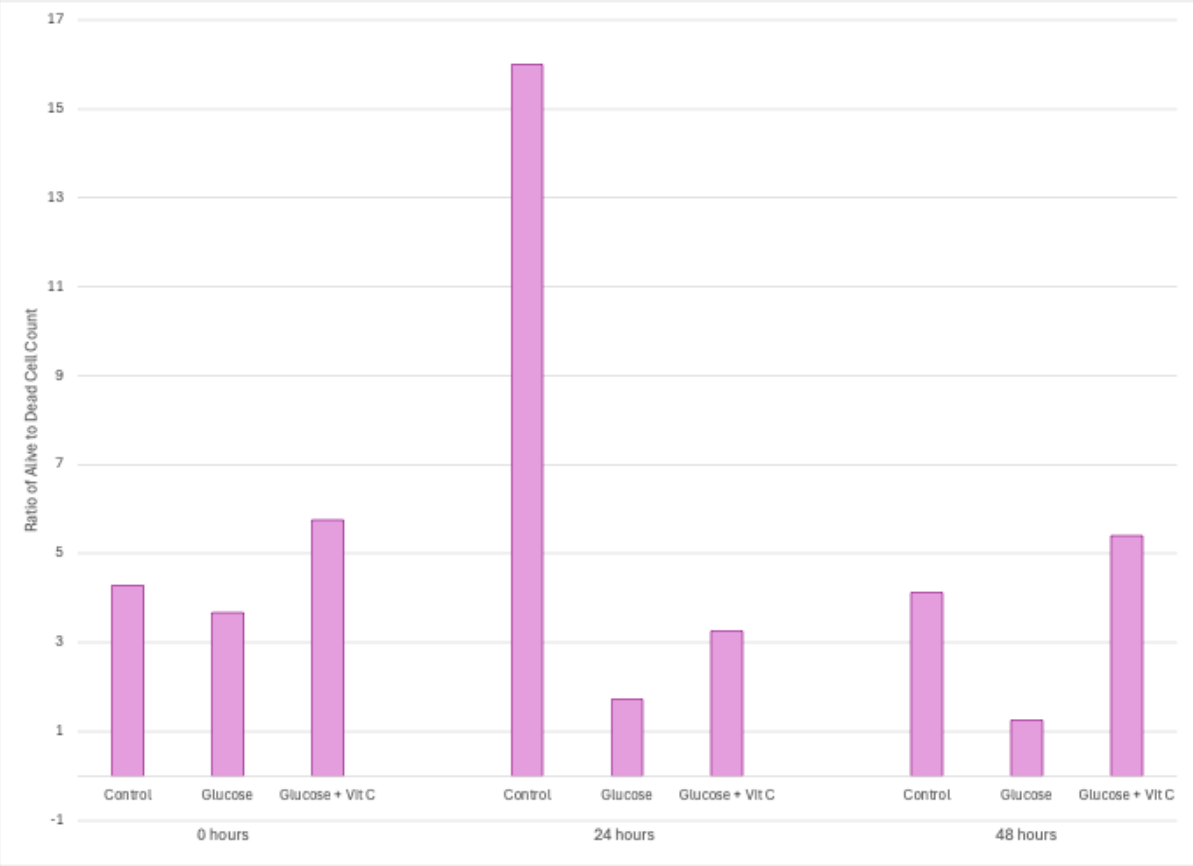

Figure 6. Ratio of alive to dead cells for control, glucose, and glucose + vitamin C flasks at 0, 24, and 48 hours.

- Control Treatment
  - Showed variable ratios of alive to dead cells between each time point due to a significant decrease in the number of dead cells after 24 hours, which then increased again after 48 hours.
- Glucose Treatment
  - Showed a significant decrease in the ratio of alive to dead cells after 24 hours, and another slight decrease after 48 hours.
- Glucose + Vitamin C Treatment
  - Showed a relatively constant ratio of alive to dead cells, with a slight decrease after 24 hours.

Results

Cell Count Analysis

- Control treatment
  - Steady increase in the number of alive cells over time, with only minor increases in dead cell counts.
- Glucose treatment
  - Higher dead cell count after 24 hours compared to the control group and had over two times fewer living cells after 48 hours.
  - Significant decline in cell survival over time, suggesting glucose promotes cell stress and apoptosis.
- Glucose + Vitamin C treatment
  - At 0 hours had highest dead cell count but contained the lowest number of dead cells after 48 hours.
  - Addition of Vitamin C provided a protective effect, with higher survival rates at 24 and 48 hours compared to the glucose-only treatment.

Results

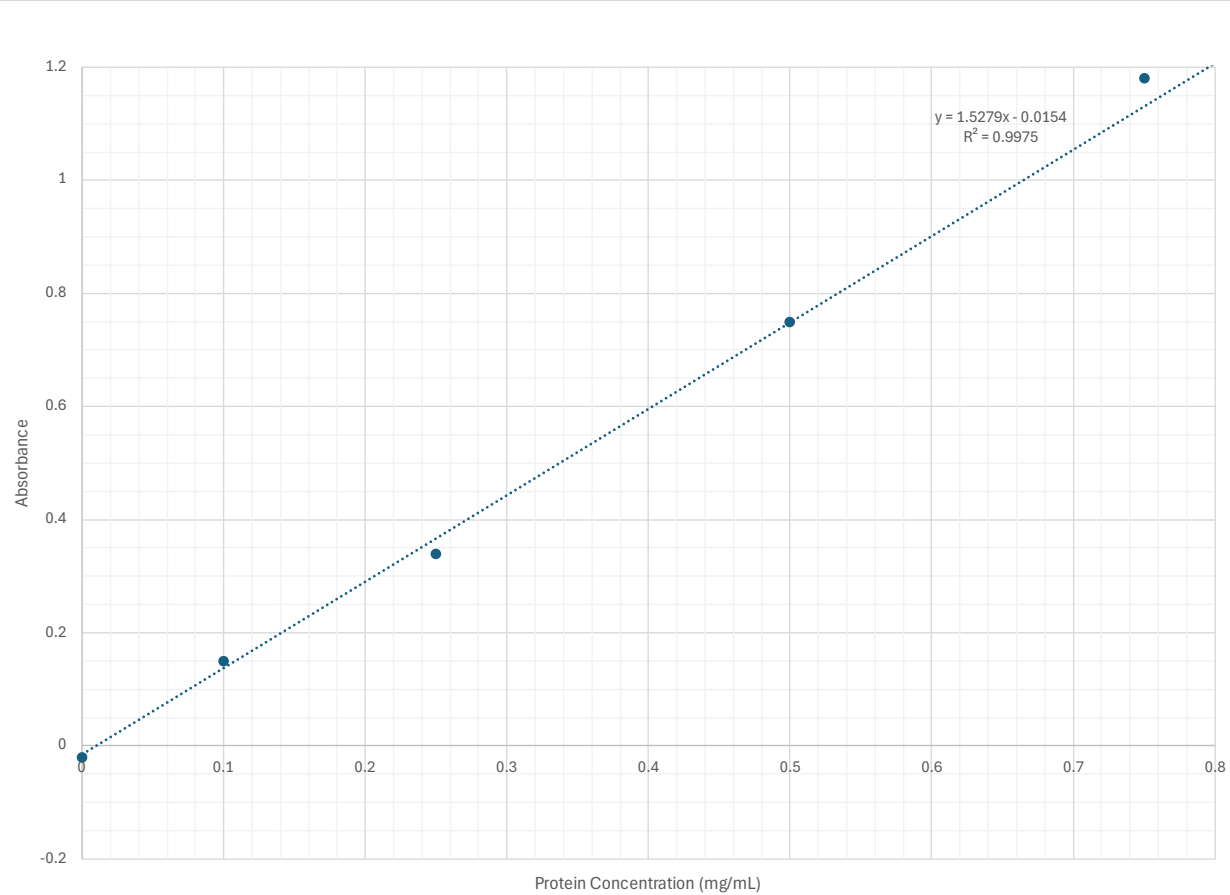

Figure 7. Standard curve for BCA protein content assay generated using Nanodrop (n=3).

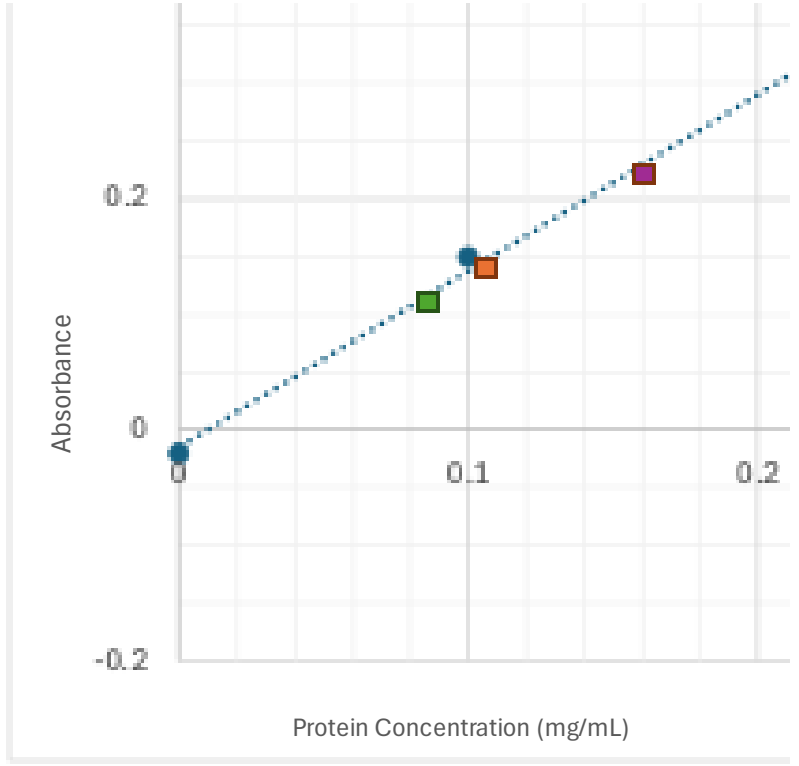

Figure 8. Zoomed in image of standard curve for BCA protein content assay showing the protein content of the control flask in purple, glucose + vitamin C flask in orange, and glucose flask in green.

Results

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Control	3	0.478	0.159333333	0.000101333		
Glucose	3	0.252	0.084	0.000651		
Glucose + Vit C	3	0.319	0.106333333	0.000529333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.008982889	2	0.004491444	10.51313394	0.01094197	5.14325285
Within Groups	0.002563333	6	0.000427222			
Total	0.011546222	8				

Figure 9. Single Factor Analysis of Variance (ANOVA) of the protein content of each flask measured using the BCA assay and Nanodrop.

- Null Hypothesis: The mean protein content of each treatment are all equal.
- F-Statistic (10.5)
  - Large F value indicates a larger difference between the group means compared to the variation within each group.
- P-Value (0.0109)
  - Since P-value < 0.05, we reject the null hypothesis, further supporting the conclusion that there are significant differences between each of the treatments.

Results

Protein Concentration Analysis

Control treatment

The highest protein content was observed, suggesting that, in the absence of glucose, cells maintain optimal protein production and viability.

Glucose treatment

Lower protein levels in the glucose treatment indicate that glucose has a detrimental effect on cell viability and protein synthesis over time.

Glucose + Vitamin C treatment

Protein concentrations were higher compared to the glucose-only flask, demonstrating the Vitamin C helps alleviate some of the negative effects of glucose on protein production.

Protein concentrations were still lower compared to the control, indicating that the addition of Vitamin C does not sufficiently counteract the negative effects of glucose to promote the same level of cell survival observed in the absence of glucose.

Main Findings

High-glucose levels induce apoptosis in endothelial cells

Ascorbic acid inhibits apoptosis of endothelial cells in the presence of excess glucose

Discussion

Physiological Mechanisms

Feng et al. (2000)

- Reactive oxidative species
- JNK pathway
- Capase-3 activation

Hsueh et al. (2020)

- PI3K/Akt pathway

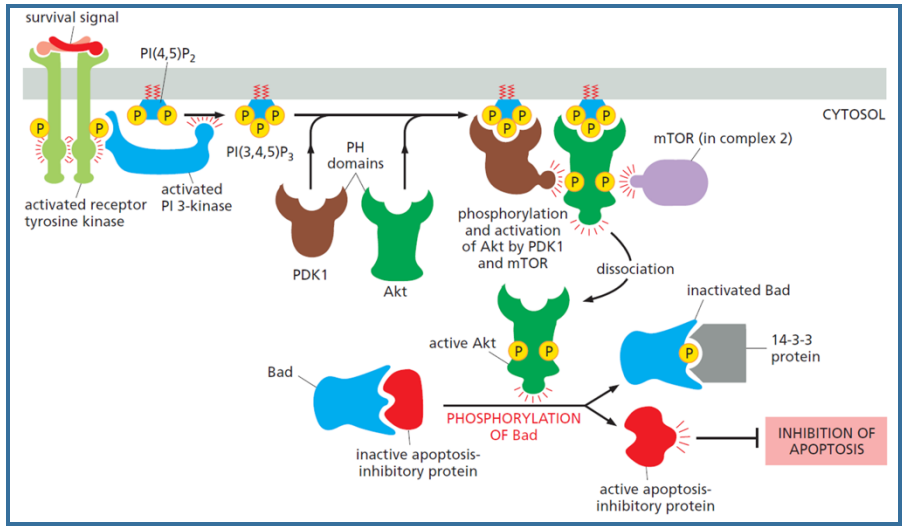

Figure 10. Signaling through the PI3K/Akt pathway (*Alberts, 2002*).

Feng et al. (2007)

- Reactive oxidative species
- Imbalance of MMP-2 & TIMP-2

Discussion

Limitations

- Limited time points
- Induced stress on the cells
- Only used one type of endothelial cell
- Limited measure of cell viability
- Protein analysis
- Vitamin C-only treatment

Discussion

Future Research Directions

- Long-term effects

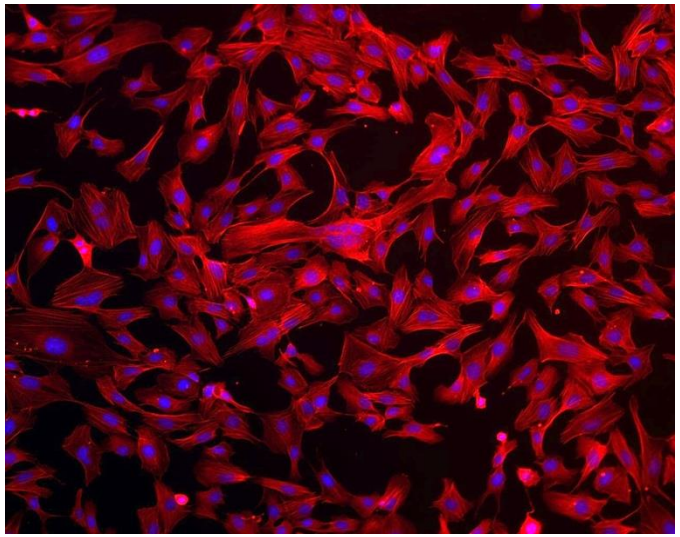

Figure 11. Stained human endothelial cells.

- Human endothelial cells
- Other cell types

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- Border image from SlidesGo.