Investigating the effect of Vitamin C on human (pre)adipocytes using RNA-Seq

Introduction

We **evolved as hunter gatherers** where our ability to **store excess energy, as fat,** was **a survival advantage** in periods of feast and famine. We are ill-equipped for the **21st Century "obesogenic" environment**, leading to **increased rates of obesity** and associated diseases, **type 2 diabetes** (T2D) and **cardiovascular disease** (CVD)[1].

In addition to serving as a vital energy store, fat tissue, which is made up of **mature fat cells** (**adipocytes**) derived from precursor cells (preadipocytes), serves as an **endocrine organ** producing and **secreting hundreds of biologically active factors**, called **adipokines**, that **regulate all aspects of our physiology** including metabolism, immune and inflammatory systems, and even our behaviour.

In **obesity**, the endocrine function of **fat becomes dysregulated**, due to intracellular **stress** and hypoxia, leading to altered adipokine production and chronic **inflammation**, which in turn promote **diseases** such as T2D and CVD [2].

Therapeutic approaches to **reduce obesity** and **prevent or reverse the dysregulation of fat** in obesity are under investigation. **This project represents part of a larger research program investigating whether vitamin C may be able to protect fat from obesity-induced dysregulation (see Fig 1).**

This *in-silico* project interrogated a **unique RNA dataset** of over 15,000 genes from a previous wet-lab study where human preadipocytes were **matured into adipocytes** over a 14-day period in the **absence or presence of vitamin C**.

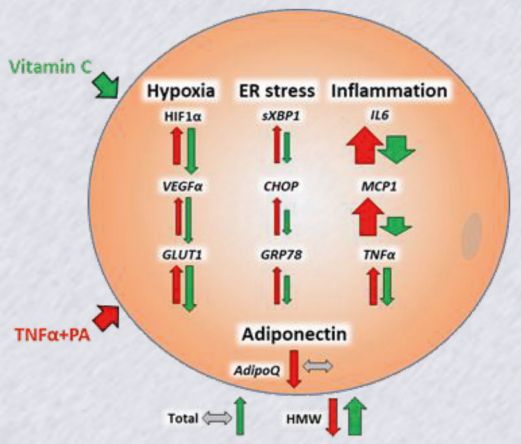

Figure 1 - Vitamin C reduces stress in fat cells. Schematic shows induction of markers of **hypoxia**, **ER stress** and **inflammation** in human fat cells treated for 24 h with a cocktail that mimics obesity (**TNFα+PA**). Co-treatment with **vitamin C reduced these adverse effects** and increased production of HMW adiponectin, an anti-diabetic, cardioprotective adipokine [3].

Hypothesis, Aims and Methods

Hypotheses: Vitamin C will:
1. Have no significant effect on adipogenesis per se (based on published reports)
2. Change expression of genes encoding proteins involved in specific processes or pathways

Aims: Screen for Vitamin C-induced changes in expression of:
1. Recognised adipogenic marker genes (n=81)
2. Genes coding for proteins involved in Sugar and/or Vitamin C transport (n=11)

Methods: GraphPad PRISM (version 9.4) was used to perform statistical analysis of Vitamin C-induced changes in gene expression and generate the Figures. A literature search was performed to interpret the findings.

Results

Changes in adipogenic marker genes

Markers of adipogenesis were altered significantly in 24 out of 81 genes, of which 23 **(96%)** supported an **increase in adipogenesis**, as per figure 2.

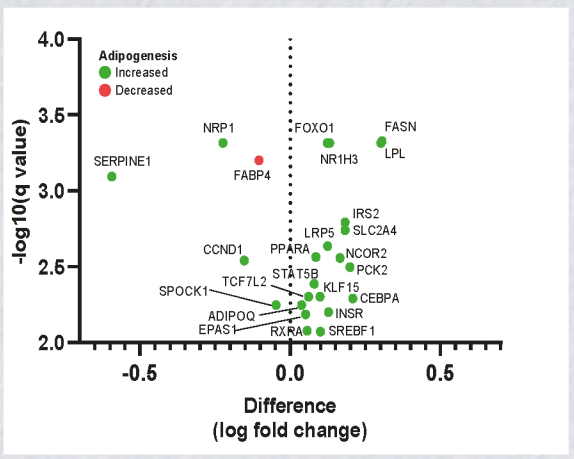

Figure 2 – Effect of vitamin C on expression of markers of adipogenesis. Volcano plot showing genes where expression was significantly altered by Vitamin C. Changes consistent with increased adipogenesis are shown in **green**. Changes consistent with decreased adipogenesis are shown in **red**.

Changes in transporter genes

Gene expression was **increased significantly** in **4 transporter genes**, notably at the 14-day timepoint, as per figure 3.

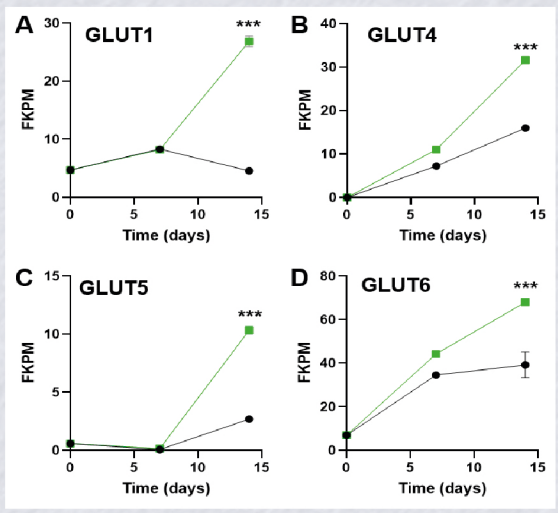

Figure 3 – Effect of vitamin C on GLUT transporters. Relative expression of (A) GLUT1, (B) GLUT4, (C) GLUT5 and (D) GLUT6 at days 0, 7 and 14 in cells matured in the absence (**black**) or presence (**green**) of vitamin C. ***p<.001 at day 14 (- vs + vitamin C)

Discussion

Increased Adipogenesis

The presence of **vitamin C** significantly altered the expression of 24 out of 81 (30%) adipogenic marker genes. Of these 24 genes, 23 (96%) were altered in a manner consistent with **increased adipogenesis**.

Upregulated Transporters

GLUT1 and **GLUT4** are able to **transport** both **glucose and vitamin C** (figure 4). GLUT1 is constitutively present at the cell membrane whereas GLUT4 resides inside the cell under basal (fasted) conditions, being transported to the cell membrane following insulin stimulation (after a meal) [4]. **Increased expression** of these transporters suggests vitamin C may **increase both basal and insulin stimulated glucose and vitamin C uptake.**

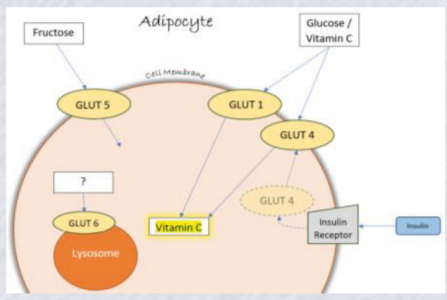

Figure 4 – adipocyte membrane transporters. Depiction of adipocyte with the 4 glucose membrane transporter (GLUTs) genes upregulated by vitamin C, showing individual known functions.

GLUT5 is a **fructose transporter**. Increased expression levels may result in enhanced **fructose uptake**. High levels of fructose can cause liver disease, therefore vitamin C may **protect the liver.**

The function of **GLUT6** is poorly understood. It resides inside the cell on the **lysosome membrane**. Lysosomes recycle redundant cell contents and influence metabolic processes, thereby potentially acting to **break down sugars** (glycolysis) [5].

Summary and Conclusions

The observations made above suggest that vitamin C:

(i) increases adipogenesis in a manner that would reduce adipocyte dysregulation and improve adipocyte function.
(ii) upregulates transporters involved in uptake of sugars (glucose and fructose) and vitamin C, that would improve adipocyte function and reduce circulating sugars.

Together, these effects would help mitigate the negative effects of obesity.

Future Work

- More detailed analysis of the molecular pathways implicated in mediating the vitamin C response.
- Characterisation of the effects of vitamin C on adipocyte function, including basal and insulin stimulated glucose and fructose uptake.
- Determine whether vitamin C supplementation is able to increase adipocyte maturation and reduce obesity-induced adipocyte-dysregulation over long periods (essentially combining the approach used in Figure 1 with the approach described here).

References

[1] Heitmann, B. L. et al., 2012. Obesity: lessons from evolution and the environment. Obesity Reviews, 13(10), pp. 910-922.
[2] Unamuno, X. et al., 2018. Adipokine dysregulation and adipose tissue inflammation in human obesity. European Journal of Clinical Investigation, 48(9), p. e12997.
[3] Luo, X. et al., 2022. Vitamin C protects against hypoxia, inflammation, and ER stress in primary human preadipocytes and adipocytes. Molecular and Cellular Endocrinology, Volume 556
[4] Rivas, C. I. et al., 2008. Vitamin C transporters. Journal of Physiology and Biochemistry, 64(1), pp. 357-375.
[5] Maedera, S. et al., 2019. GLUT6 is a lysosomal transporter that is regulated by inflammatory stimuli and modulates glycolysis in macrophages. FEBS Letters, 593(2), pp. 195-208.

Student: Leigh Johns

Supervisor: Jon Whitehead

UNIVERSITY OF LINCOLN
Lincoln Academy of Learning and Teaching

Undergraduate Research Opportunities Scheme

DISCOVER_
LNCN.AC/UROS
@UOL_LALT
#UROS2022