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

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Abstract

During this research I studied the effects of vitamin C on fat cells (adipocytes) and response mechanisms through analysis of key genetic markers from a prior wet-lab experiment dataset. Understanding how vitamin C affects adipocyte maturation and cell transport is relevant to the field of obesity and disease prevention. Results indicated that vitamin C significantly altered gene expression in support of adipocyte creation (adipogenesis), and enhanced vitamin C and sugar uptake and storage. This project provided a unique experience and opportunity for student involvement, bridging the educational gap during the summer period, and contributing to the field of human health.

Keywords: *Adipocyte, preadipocyte, adipogenesis, vitamin C, obesity*

Introduction

During the summer of 2022, funded by the University of Lincoln Undergraduate Opportunities Research Scheme (UROS), an *in-silico* research project was undertaken, with a supervisor from the School of Life Sciences. The project analysed a transcriptomic (RNAseq) dataset from a previous wet-lab study, which catalogued how individual genes responded and altered their expression levels. The focus was the extent to which human immature fat cells (preadipocytes) were differentiated (adipogenesis) into mature fat cells (adipocytes) in the presence of vitamin C. Analysis involved in depth visualisation of individual gene expression levels in combination with current literature. The project focused upon key gene subsets relevant to adipogenesis and vitamin C uptake into the cells. The Covid-19 restrictions provided a setting conducive to data analysis research, as opposed to further wet lab studies, however this was beneficial providing time to pause and reflect on the data and to shape future research through a greater understanding of key mechanisms and responses.

Background/ rationale for the session

Obesity is a known risk to health and directly leads to a lower life expectancy through type-2 diabetes, cardiovascular disease, cancer and many other associated morbidities (Walls, et al., 2012) (Chatterjee, et. al., 2020). A study by Vidra *et al.* showed that across 27 European countries and the USA between 1975 and 2012 increasing obesity among populations led to a notable reduction to increases in life

expectancy by an average 0.78 years for men and 0.3 years for women (Vidra, et al., 2019). Future projections by Keaver *et al,* - based on data from current adolescent trends show morbid obesity levels in the UK are expected to rise significantly to 5% in Scotland, 8% in England and 11% in Wales by 2035, totalling 5 million people, leading to further reductions on life expectancy and increased pressure on public health services (Keaver, et al., 2018).

With an interest in human ageing and disease, this project assisted in gaining valuable skills and knowledge for my career aspirations. My aim was to assist in shaping future research through my contribution providing potential benefit to human health and disease. Being involved in such a project over the summer kept me actively involved in learning, boosting both my knowledge, and maintaining academic momentum which are important in student performance and success (Kuhfeld, 2019).

Review of literature

Excess lipids are stored through two mechanisms within adipocytes – hyperplasia (adipogenesis – creation of new cells) and hypertrophy (cell expansion), and the pathway selection between them is regulated by a multitude of environmental and genetic factors. Obesity, especially involving high levels of visceral deposition, is a key driver of hypertrophy instead of hyperplasia through the dysregulation of adipose signalling (Blüher, 2009). As a result, hypertrophic adipocytes lose effectiveness as an endocrine organ, and their metabolic dysregulation can lead to fibrosis, inflammation, hypoxia, and disease within the body (Ghaben & Scherer, 2019). Adipocytes release many signalling molecules (adipokines) into the bloodstream, an example of which is adiponectin which has been shown to positively reduce the risk of diseases such as atherosclerosis, type-2 diabetes, and chronic inflammatory conditions (Choi, et al., 2020).

Vitamin C has been shown to lessen inflammation and oxidative stress, presenting a potential pathway for treatment (Totan, et al., 2019). Other studies have also shown that vitamin C plays a crucial role in cellular function, evidencing an increased physiological requirement for it in type-2 diabetes and obesity (Wilson, et al., 2017). Interestingly there is also a negative correlation between vitamin C intake and the occurrence of obesity and related diseases, suggesting a potential causative link with low dietary levels and further supporting its role in disease prevention (Garcia-Diaz, et al., 2014).

Literature from Kim *et al.* was conflicting on the effects of vitamin C on adipogenesis, with some studies supporting positive correlation (Kim, et al., 2013) and some showing inhibition (Rahman, et al., 2014). The dataset for this research included over 15,000 genes, of which over 2,000 genes showed altered levels of expression in the presence

of vitamin C during a 14-day period of differentiation. Therefore, this unique dataset presented an opportunity for the first time to study the changes at the RNA expression level.

Methodology

Statistical analysis and visualisation of the dataset was conducted with the focus of the research being to identify whether vitamin C had a positive effect on adipogenesis, and whether cellular mechanisms were altered by its presence. Despite evidence showing the positive effects of vitamin C in mitigating the detrimental health effects of visceral adiposity, there was still a gap in the understanding of the individual genes and pathways involved (Marks, 2021). Initial steps in the project involved literature research to increase understanding of the genes involved in adipocyte function. Subsets of genes from the data were then analysed using the statistical analysis and visualisation software GraphPad Prism in coordination with literature on adipose tissue and vitamin C to facilitate interpretation.

Results

Specific to adipogenesis, 24 out of 81 identified genetic markers demonstrated significant changes in expression in the presence of vitamin C when compared to the control sample. Of these, 23 genes (96%) supported increasing adipogenesis. Furthermore, genes coding for four vitamin C/sugar transporter genes were also upregulated by vitamin C supplementation, especially at the 14-day point. These results support that vitamin C improves adipocyte function and can increase vitamin C and sugar uptake into the cells.

Discussion

Key Areas of Development

This project helped me develop my understanding of cellular mechanisms, especially in the field of human health relating to dietary and lifestyle factors. Having a specific project format provided an opportunity to dissect literature on an area of interest discovering how complex gene expression can be in response to the addition of a single vitamin. This project has granted me a deeper comprehension and interest in the coordinated nature of cellular biochemistry and genetics. Being able to work with direct academic supervision from a specialist in the field has provided me with greater confidence that with time, further study, and dedication, I have the potential to make a difference to future research projects. This is an area of personal aspiration.

I also feel that maintaining my learning journey throughout the summer break will be beneficial in maintaining learning momentum and reducing knowledge loss. Using Graphpad Prism for statistical analysis and visualisation of data has been a new

experience for me, yet I have found the software to be easier to learn and use than some of the software packages previously used. It is a highly flexible tool with a more natural visual layout that felt more synergistic with my previous experience of using Excel.

By its nature, the subject material has proven challenging to learn and understand. Initial project weeks were spent solely reading the literature to obtain sufficient understanding of adipose biochemistry, and even then, it was specific only to the changes in vitamin C response within limited mechanisms as exhibited through changes in RNA expression. The knowledge journey continued throughout the project as new links and areas of significance were discovered. The greatest limitation was having sufficient time to interrogate the data in full as there was vast detail and many potentially undiscovered mechanisms worthy of further investigation, which is why specific hypotheses were set during project commencement to prevent a drift in focus. My report writing skills have also been challenged. Having the experience of academic writing, I have discovered that brevity and concision are paramount in relaying findings in a more professional setting as a researcher, without losing the context of the content being discussed. This is an element of my development that requires further improvement despite gains made throughout the project. To further develop myself in retrospective view of the gains and challenges presented by this project, I require the allocation of more time and devotion to study and academia. By doing so I would greatly improve my future career prospects whilst further aiding my confidence and skills as a potential future researcher.

Challenges and Lessons Learnt

The UROS project has been a challenging yet positive experience. It has given me the perfect vehicle for developing my knowledge base in an area of interest as well as providing a great learning opportunity for some of the requirements of professional research publication. The time commitment required for the project involved daily study, but having a clear project plan from the outset, incorporating weekly meetings and fluid deadlines, has ensured that it remained on track yet with the flexibility to adapt to unforeseen events and new research findings. The personal gains I made during this project will stand me in good stead for future projects and even helped in formulating ideas and accumulating relevant knowledge towards my year-3 research project. I have learnt to persist when hurdles present themselves, even when the project appears to be off-schedule, or the content becomes difficult to fully comprehend. The support of a supervisor has been a vital experience along the journey – being able to seek clarity, guidance and advice when needed.

Conclusion

I would recommend the UROS project journey to any student who has the time and desire to improve their academic performance and boost their future career prospects. The gains are diverse and the continuation of education through the summer period can only help further the momentum of the learning process. The opportunity to work closely with a supervisor on an area of current research is an advantage that few students experience and should be aspired to as part of the developmental journey.

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