Validating the efficacy of mitochondrial biogenesis compounds

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Introduction

Mitochondria are organelles that perform various metabolic processes especially the production of adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS). Inborn genetic mutations affecting OXPHOS are known as mitochondrial diseases. As mitochondrial diseases affect ATP production, tissues that demand high energy such as the heart, brain and skeletal muscles have the potential to be affected (1).

Currently, there are no effective treatments for mitochondrial disorders. However, there have been a few reports claiming that several compounds have effects on increasing the number of mitochondria present, which is known as mitochondrial biogenesis, as well as increasing oxygen consumption of the mitochondria. This potentially could have beneficial effects for mitochondrial disease patients. These compounds have either been used to treat other diseases or new compounds altogether (2).

Aim

This project aims to confirm, or refute, these claims by assessing the cell area containing mitochondria after dosing cells in the compounds shortlisted.

Methodology

1. Fibroblast cells were dosed for 48 hours with varying concentrations of compounds of interest, listed below, which were chosen from current literature (2).
   a) Acipimox
   b) Bezafibrate
   c) AICAR
   d) Nicotinamide Riboside (NR)
   e) Quercetin
   f) Mitochondrial Division Inhibitor 1 (M-DIVI 1)
2. The cells were stained and imaged using Cell Discoverer 7 microscope. The images were analysed with Columbus software and visualised TIBCO Spotfire. A brief outline of the Columbus pipeline is shown in Figure 1 below.
3. Basal oxygen consumption levels were tested using Seahorse Extracellular Flux analysis for Acipimox due to promising data obtained in this project.

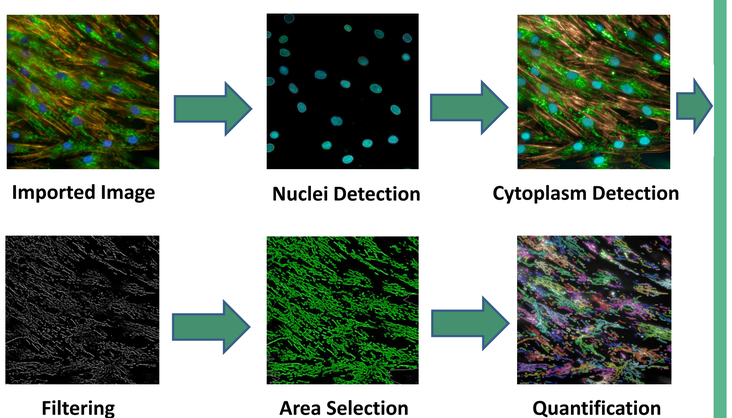
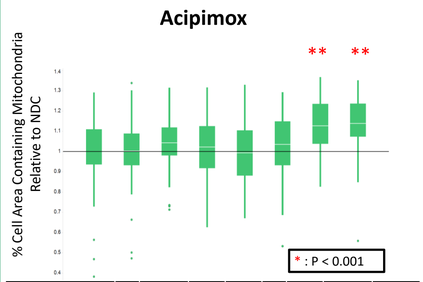


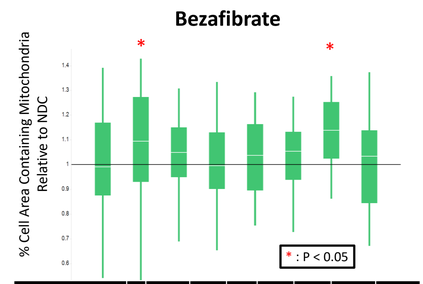
Figure 1 shows the pipeline used in Columbus quantification software to extract data from immunofluorescent images.

Results



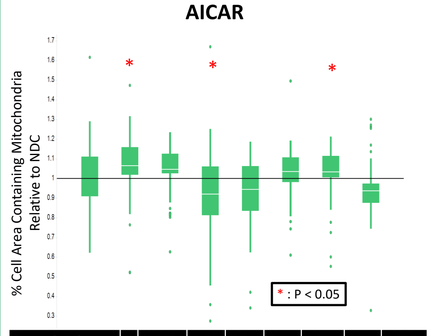
Conc. (uM)	0	0.001	0.01	0.1	1	10	100	1000
Percent Change	0	0	+4.2	+2.1	-1	+3.5	+12.7	+13.8

Figure 2 shows statistical significance at concentrations of 100uM and 1mM



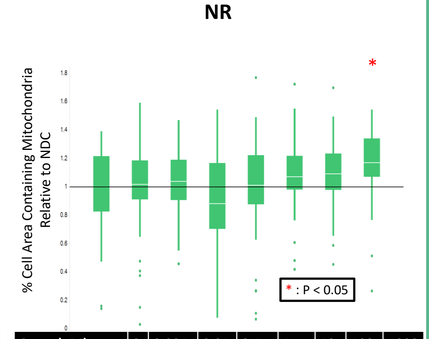
Conc. (uM)	0	0.001	0.01	0.1	1	10	100	1000
Percent Change	0	+8.5	+4.7	0	+3.6	+4.2	+13.4	-2.3

Figure 3 shows statistical significance at concentrations of 1nM and 100uM



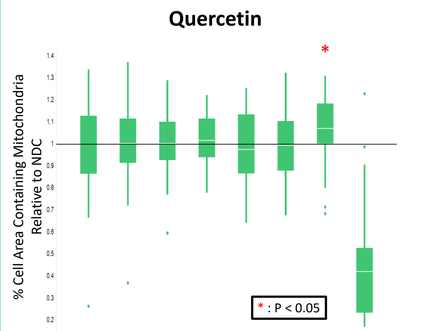
Conc. (uM)	0	0.001	0.01	0.1	1	10	100	1000
Percent Change	0	+6.7	+4.6	-8.0	-5.6	+3.5	+3.4	-6.7

Figure 4 shows statistical significance at concentrations of 1nM, 100nM and 1mM



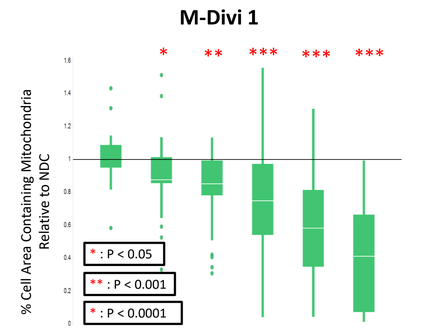
Conc. (uM)	0	0.001	0.01	0.1	1	10	100	1000
Percent Change	0	+1.8	+3.9	-12.7	+1.3	+7	+9.4	+17

Figure 5 shows statistical significance at concentration of 100uM



Conc. (uM)	0	0.001	0.01	0.1	1	10	100	1000
Percent Change	0	+0.3	+0.3	+1.8	-0.2	-0.1	+7.3	-42.2

Figure 6 shows statistical significance at concentration of 100uM



Conc. (uM)	0	0.001	0.01	0.1	1	10
Percent Change	0	-12.2	-14.8	-25.1	-41.7	-58.7

Figure 7 shows statistical significance at concentrations of 1uM, 10uM, 100uM and 1mM

Discussion

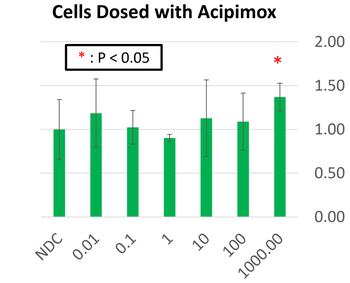


Figure 8 shows basal oxygen consumption rate of cells dosed in acipimox

It is thought that acipimox increases mitochondrial biogenesis as an NAD+ precursor (2). Figure 2 shows an increase of 12-13% in mitochondrial biogenesis at 100uM and 1000uM respectively. It is further noted that Seahorse analysis shows that the oxygen consumption levels are higher at 1000uM. On the other hand, bezafibrate is a peroxisome proliferator-activated receptor (PPAR) agonist, which is said to stimulate PGC-1α. This supposedly indirectly increases mitochondrial biogenesis, which is shown in Figure 3.

It is suggested that AICAR increases mitochondrial biogenesis as it forms an adenosine monophosphate (AMP) mimetic. This binds AMP kinase, triggering a signalling cascade to cause the cell produce more mitochondria, thus more ATP (2).

Furthermore, NR is an NAD+ precursor, like acipimox. From Figure 5, the percentage cell area containing mitochondria increased at the concentration of 100uM. Quercetin is a polyphenol that acts on the mitochondrial membrane and is said to increase mitochondrial biogenesis (3). According to Figure 6, its effect is shown at 100uM, as there is an increase in cell area containing mitochondria. However, at concentration of 1000uM, the cells died abruptly which suggests that quercetin may be toxic to cells at high concentrations. Moreover, M-Divi 1 is a mitochondrial fission inhibitor. It has been included as a negative control for the other experiments. Interestingly, cells started to die more at higher concentrations, which why data of 100uM and 1000uM was excluded from the graph (4).

Limitations of this project include the lack of cells to carry out the experiment as the fibroblasts take some time to grow to the counts required for high throughput work. Increasing cell culture capacity would have helped with this minor issue. Furthermore, not all the samples could be analysed using Seahorse due to the short nature of the project - only acipimox was chosen as this was the most promising compound to investigate.

Conclusion

Various laboratory groups have tested many compounds for mitochondrial effects and this has resulted in conflicting literature. This project tested multiple compounds supposedly linked to mitochondrial biogenesis. From this data, only acipimox appears to have a positive effect.

References

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Acknowledgements
I thank my supervisors, Gareth Ettridge and Dr Oliver Russell for their guidance during the project. Funding for this project was provided by Newcastle University Undergraduate Research Scholarships.