Investigating changes in protein levels from patients with mitochondrial myopathies after resistance exercise training.

Millicent Rice*
190342935, Biomedical Genetic Student, m.rice2@newcastle.ac.uk

MITO Let's find a cure
www.newcastle-mitochondria.com
@MitoResearch

Introduction

Mitochondrial myopathies are a group of neuromuscular disorders that are currently incurable, making symptomatic therapies a priority in research. Previous investigations have found that resistance exercise training could be a possible form of therapy (1). This has led to additional studies into patient's skeletal muscle samples pre- and post-exercise, to try and undercover the mechanism of benefit (2). In a previous RNA sequencing study of a mitochondrial myopathy cohort, one protein found to have changed levels was Nicotinamide N-methyltransferase (NNMT) (2). This project looked to investigate further the possible correlation between the protein level of NNMT and mitochondrial deficiency.

Aims

- Use quadruple immunofluorescent staining to visualise NNMT levels within skeletal muscle fibres.
- Analyse results from pre- and post-exercise samples to determine if there are significant correlations between the target protein, NNMT, and mitochondrial dysfunction.

Methods

Identification of protein targets. → Practise quadruple immunofluorescent staining for NNMT and analyse for success. → Quadruple immunofluorescent staining on patient cohort samples. pre- and post-exercise samples for each patient with controls. → Bioimaging of the stained sections.

Reason for protein target inclusion

- NNMT = Target protein
- SDHA = Mitochondrial mass marker
- Laminin = Skeletal muscle fibre membrane
- ND2 = Mitochondrial complex I marker
- Hoechst = Nuclei marker

Analysis

Sections were imaged using the Zeiss Axioscan 7 microscope at 20X magnification. Imaged sections were then segmented. An in-house tool called Quadimmuno, using the Laminin membrane marker (seen in Figure 1) was used to identify skeletal muscle fibres. Suitable skeletal muscle fibres were selected for quantification and statistical analysis.

Background

Mitochondria

Mitochondria are intracellular organelles present in cells throughout the human body. Mitochondria produce cellular energy in the form of adenosine triphosphate (ATP) by oxidative phosphorylation in the electron transport chain, composed of complexes I-V. In high energy demanding tissues, such as skeletal muscle, mitochondria functionality is imperative.

Mitochondrial myopathies

Mitochondrial myopathies are progressive adult-onset muscle conditions that primarily affect skeletal muscle (2). Symptoms of mitochondrial myopathies vary and can be multisystemic. Symptoms can include impairment of oxidative phosphorylation; skeletal muscle fibres with improper functionality and increased levels of fatigue and muscle weakness.

Treatment

Variability in the genetic cause, symptoms, severity and the multisystemic nature has made the path to discover a treatment difficult.
Studies have been conducted into the benefit of resistance exercise training to stimulate muscle regenerative capacity in mitochondrial myopathy patients. The results of the studies found increased muscular strength, improved muscle fibre regeneration and improved oxidative capacity (1,2).

Quadruple immunofluorescent staining results

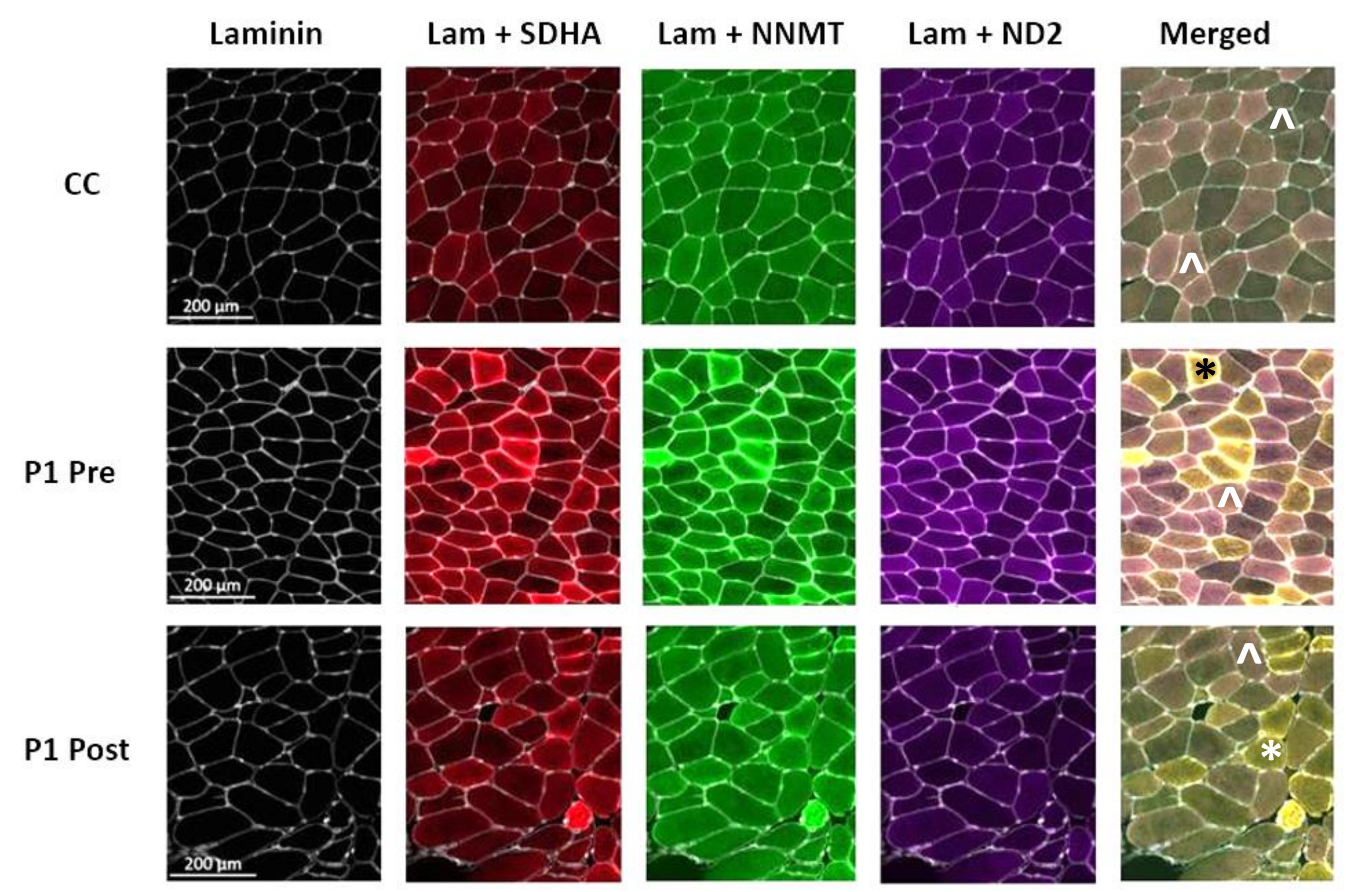

Figure 1. Representative quadruple immunofluorescent images of pre- and post-exercise samples after resistance exercise training compared to control muscle. The overlay is shown in the last column where all channels are merged. Scale bar 200µm. Key: * = Fibres with mitochondrial dysfunction, ^ = Fibres with mitochondrial functionality.

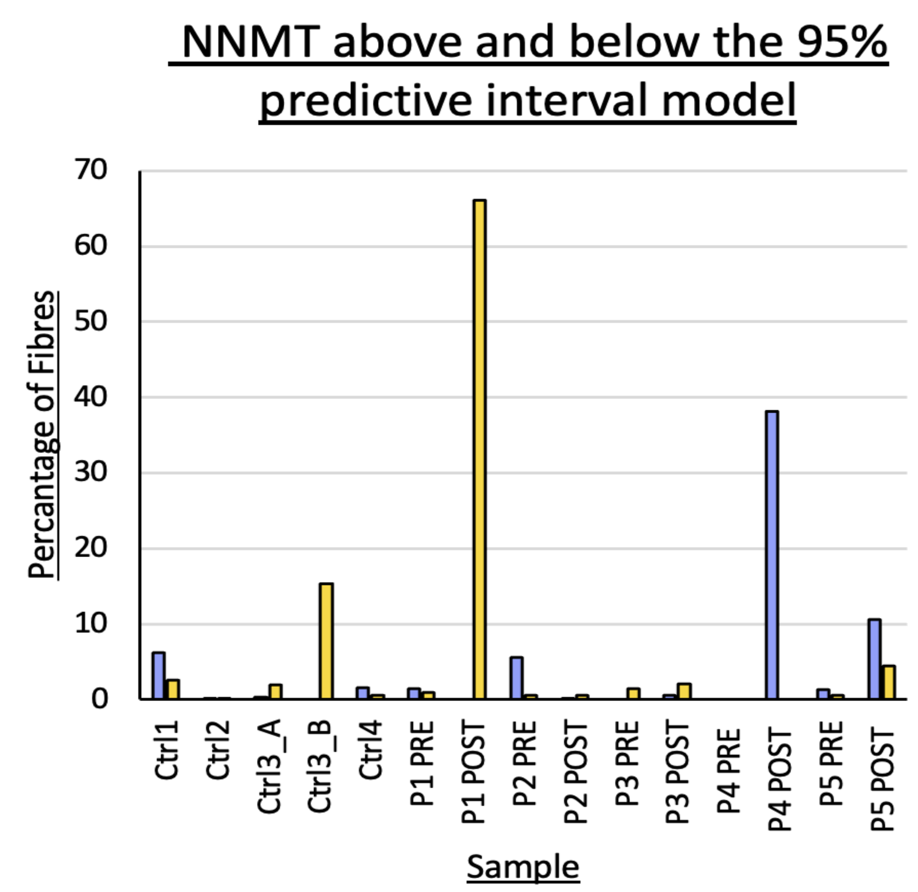

Figure 2. Representative Bar Chart of the percentage fibres with NNMT levels above (Yellow) and below (Purple) the 95% predictive interval model

- Figure 1 illustrates that NNMT localised within the skeletal muscle fibres. It was present in both patient pre- and post-samples, as well as controls.
- Figure 2 highlights that the percentage of fibres with NNMT levels above and below the 95% predictive interval model varied between patients and controls.

Statistical analysis

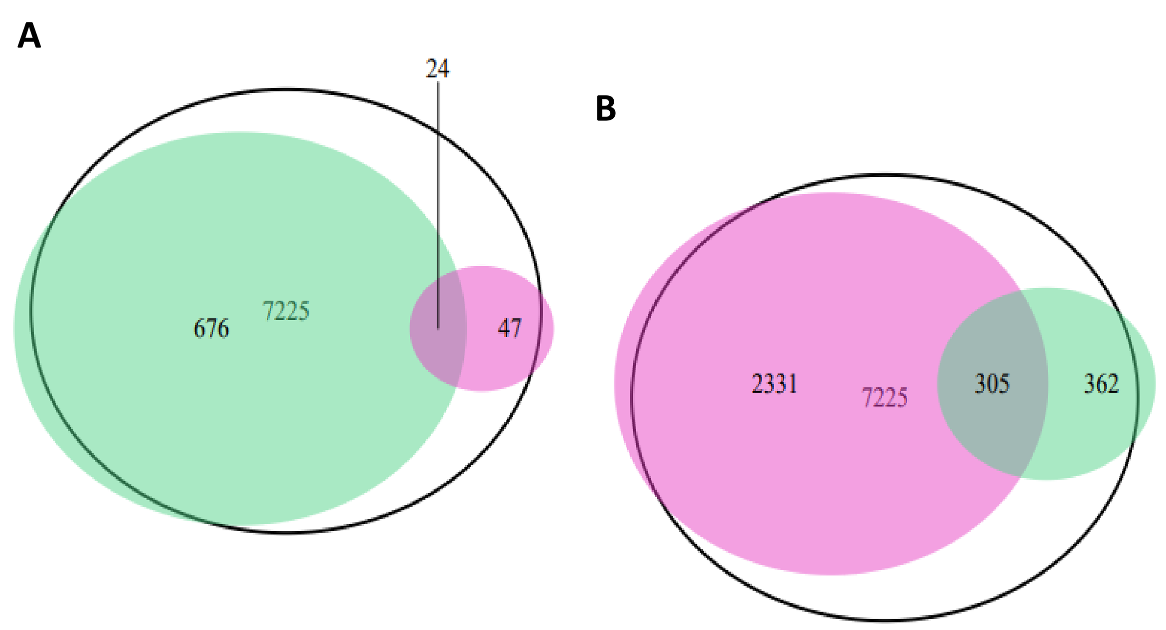

Figure 3. Venn diagram representing the number of skeletal muscle fibres with ND2 (pink) and NNMT (green) levels above (A) and below (B) the calculated 95% predictive interval model.

Sample	P value fibres above	P value fibres below
P1 Pre	1.000	0.011
P1 Post	0.007	1.000
P2 Pre	0.035	0.140
P2 Post	0.000	1.000
P3 Pre	1.000	1.000
P3 Post	0.021	0.180
P4 Pre	1.000	1.000
P4 Post	1.000	0.001
P5 Pre	0.000	0.040
P5 Post	0.056	0.008

Table 1. P values calculated from correlation data between NNMT and ND2 values above and below the 95% predictive interval model.

- The programming language R was used to analyse the correlation between protein levels of NNMT and ND2. Figure 3 and Table 1 were produced as a result.
- Values in Table 1 closer to zero indicate a significant correlation between the level of NNMT and ND2 in that sample.

Conclusions

- It is exciting to see from the results that P1, P3 and P5 have increased levels of NNMT post-exercise. This may indicate a link between resistance exercise training and the level of NNMT in patients with mitochondrial myopathies.
- Reviewing the correlation analysis, it suggests there is a connection between mitochondrial deficiency and the protein NNMT.
- This is compelling evidence for further study into NNMT, which could provide insight into the mechanism resulting in the benefits seen in patients after resistance exercise training.

Acknowledgments

Dr Amy Vincent – Project supervisor
Dr Valeria Di Leo – Project supervisor
Faculty of the Wellcome Trust Centre for Mitochondrial Research
Newcastle University Bioimaging unit
Newcastle University Summer Scholarship fund

References

1) Murphy, J. et al. 2008. *Resistance training in patients with single, large-scale deletions of mitochondrial DNA* | Brain | Oxford Academic
2) Di Leo, V., 2022. *Understanding the effects of resistance exercise training on mitochondrial dysfunction in mitochondrial myopathy and myotonic dystrophy type 1 patients*. Newcastle University, PhD thesis.

The Newcastle upon Tyne Hospitals NHS Foundation Trust
Newcastle University
wellcome centre mitochondrial research
NIHR | Newcastle Biomedical Research Centre