uOttawa
Faculté de médecine
Faculty of Medicine

UCP3 Protein Expression and Mitochondrial Content in UCP3 KO and WT Mice

Kijoo Kim, A. Brianne Thrush, Mary-Ellen Harper
Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

Introduction

Uncoupling protein 3 (UCP3) is an inner mitochondrial protein responsible for proton leak and may play a key role in facilitating complete fatty acid oxidation. Acylcarnitines are the byproducts of incomplete fatty acid oxidation and have been shown to increase oxidative stress and have been linked to the development of insulin resistance in this tissue. The Harper Laboratory has investigated the effects of acylcarnitines on mitochondrial respiration in UCP3 knockout and wild type mice. Preliminary research has demonstrated that mitochondrial respiration in white gastrocnemius is decreased in the presence of long-chained acylcarnitines compared to short-chained acylcarnitines and UCP3 may play a role in this (Koves *et al*, 2007).

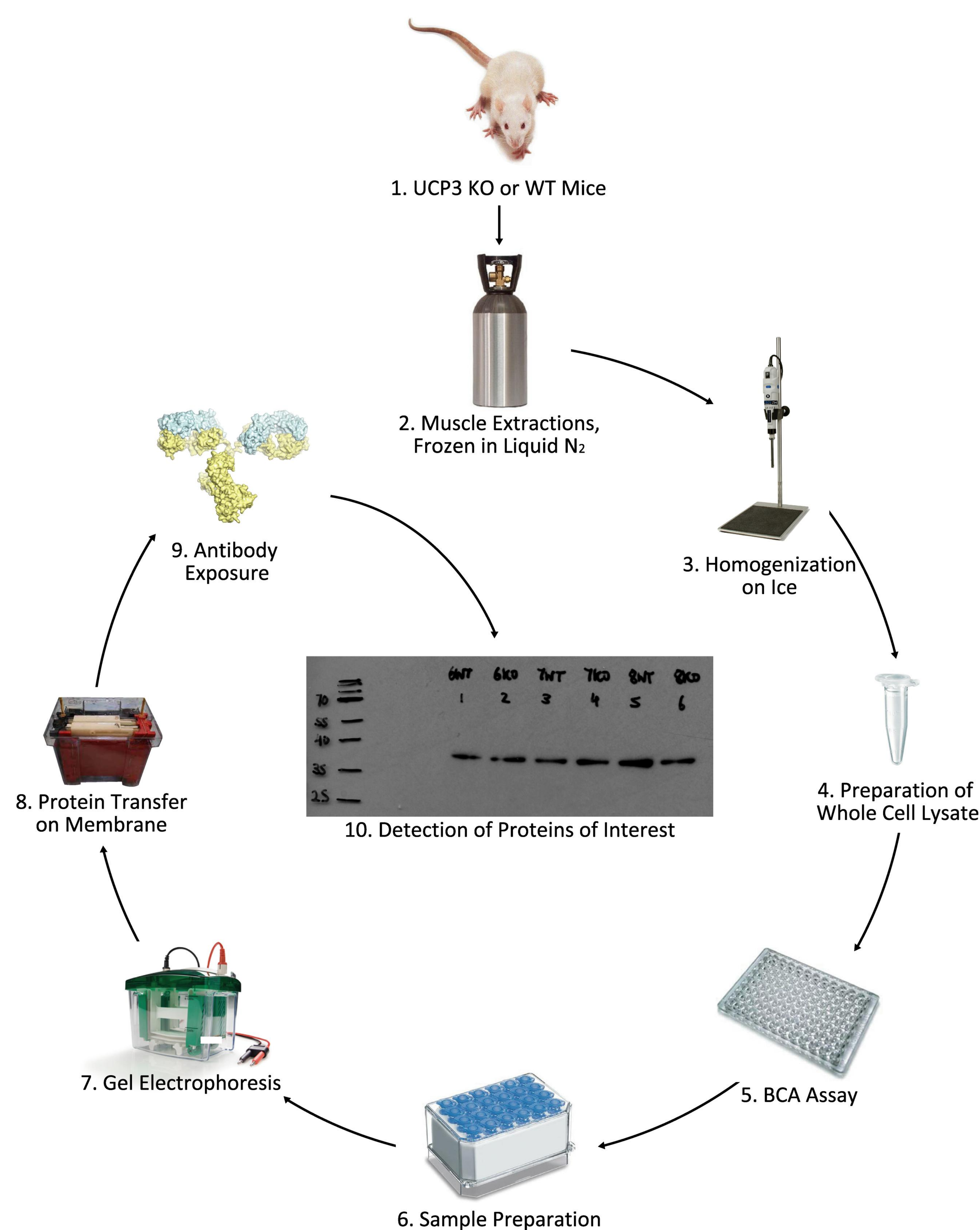
Purpose

The purpose of this research is to assess the expression of key protein from white gastrocnemius muscle samples of UCP3 knockout (KO) and wild-type (WT) mice. Proteins that will be measured include UCP3 and key proteins in the electron transport chain.

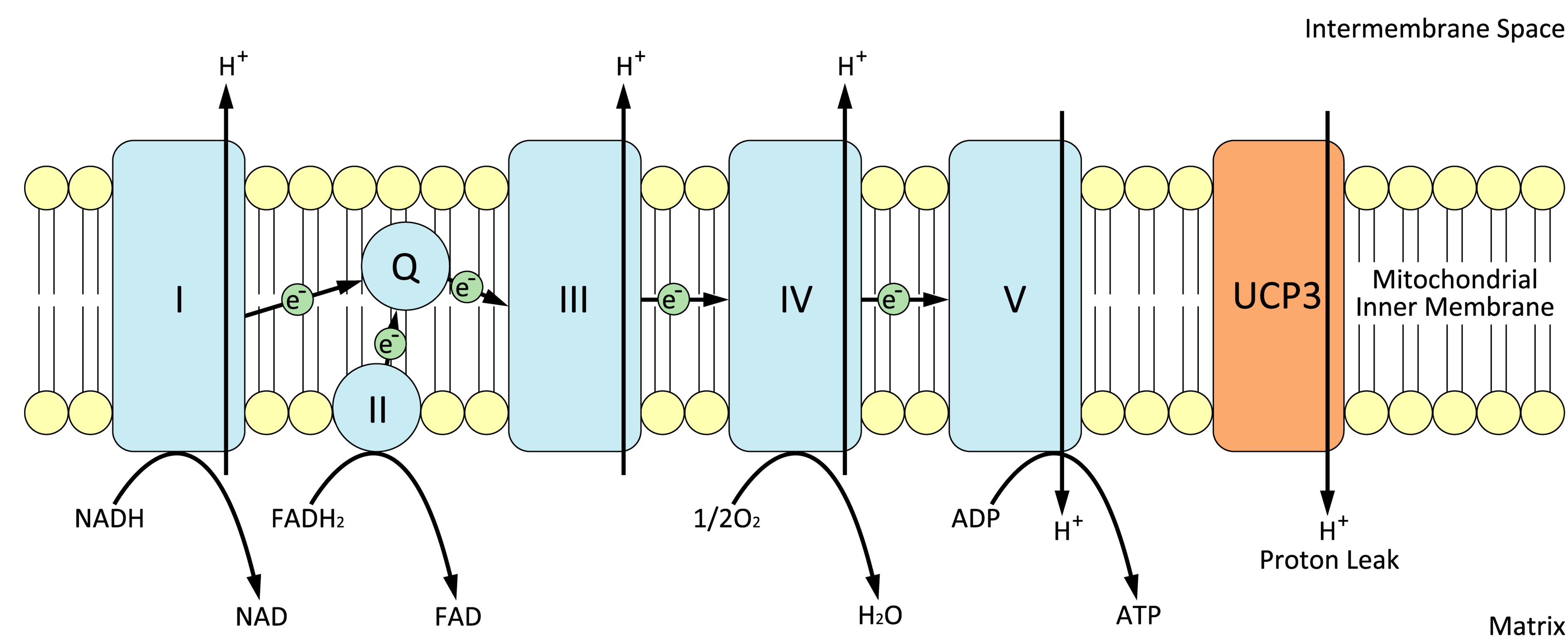
Hypothesis

It is hypothesized that these protein expressions take part in the formation of acylcarnitines, thus linking the inefficiency in fatty acid oxidation.

Methods



Protocols followed to detect UCP3 mitochondrial content in UCP3 KO and WT Mice. UCP3 KO and WT white gastrocnemius muscle samples (1) were collected with the use of liquid nitrogen and kept at -80ºC (2). The collected samples were homogenized (3), sonicated, and centrifuged at 1500xg (4). Bicinchoninic acid (BCA) assay (5) was performed to determine various protein concentrations, contents, and the loading volume, which was determined to be 32µL, for western blots. Samples were prepared in loading buffer (6), proteins were separated on 12% polyacrylamide gel using gel electrophoresis (7), and transferred onto nitrocellulose membranes (8). Depending on the protein in question, various types and concentrations of primary and secondary antibodies (9) were exposed on the membranes to allow binding that is required for detection of the western blots (10). Figure 1A used primary antibody 1:2000 5% BSA in TBS and secondary anti-rabbit antibody 1:10000 5% skim milk in TBS. Figure 1B used 1:3000 5% BSA in TBS and secondary anti-mouse antibody 1:3000 5% skim milk in TBS. Figure 2A used 1:2000 5% BSA in TBS and secondary anti-mouse antibody 1:4000 5% skim milk in TBS.



The role of UCP3 in the electron transport chain (ETC). NADH and $FADH_2$, both produced via citric acid cycle, are oxidized to donate electrons required to run the ETC. The flow of electrons drives the movement of protons from the mitochondrial matrix to the intermembrane space to complexes I, III, IV, and ubiquinone (Q). Complex V returns protons back into the matrix, creating ATP from ADP. In contrast, UCP3 returns protons back into the matrix without any production of ATP. This proton leak leads to waste in biochemical energy, decreasing the proton gradient across the membrane and lowering ATP synthesis (Mailloux and Harper, 2011).

Results

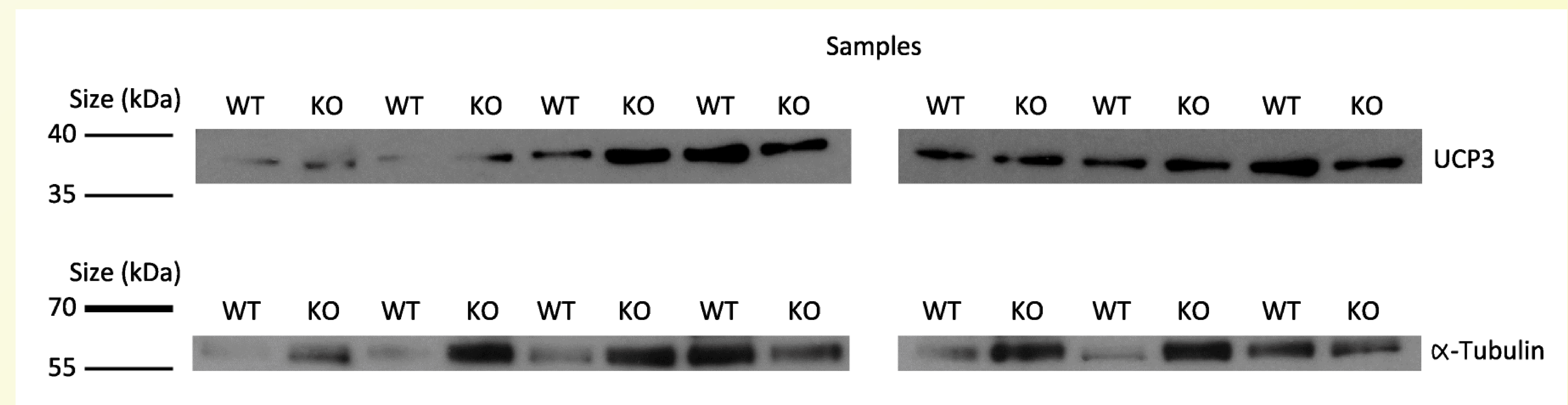


Figure 1. UCP3 protein content in UCP3 KO and WT mice. Representative western blot of UCP3 protein expression in white gastrocnemius of UCP3 KO and WT mice. α-tubulin was required to act as a loading control. N = 7. KO, knockout; WT, wild-type.

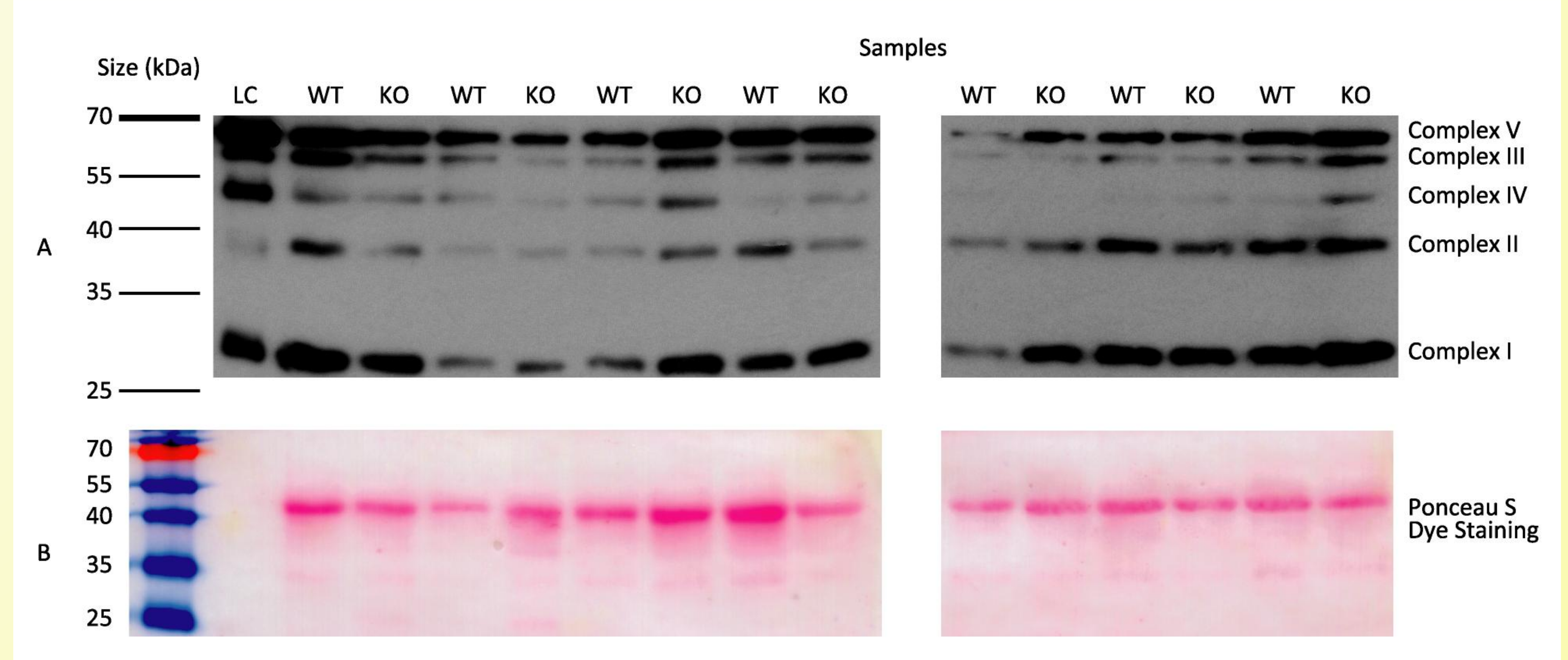


Figure 2. Expression of mitochondrial electron transport chain proteins in UCP3 KO and WT mice. Figure A. Representative western blot of mitooxphos cocktail containing five different protein expressions in white gastrocnemius of UCP3 KO and WT mice. N = 7. KO, knockout; LC, loading control; WT, wild-type. Figure B. Ponceau S staining used to assess protein loading from Figure A.

Summary

- Based on the protein expressions shown on the western blots, mitochondrial content does not appear to be different between UCP3 KO and WT mice (Figure 1).
- There is considerable variability in UCP3 expression between UCP3 KO and WT mice. UCP3 WT protein expression was thought to be greater than UCP3 KO protein expression, but the blots demonstrate inconsistent pattern. The variability in the α-tubulin blot suggests unequal protein is present on the membrane.
- Similar variability was shown in protein complexes I-V (Figure 2). To assess the protein loading, ponceau S stain was applied on the membrane to compare with the detected blot.
- Without strong evidence via quantification of protein expressions, the effects of these protein expressions on acylcarnitines were found to be inconclusive.

Future Direction

- Densitometry of mitochondrial UCP3 and ETC protein expressions will be performed using Image J software. A T-test will be utilized to assess the difference in protein expression between UCP3 KO and WT mice.
- The protein expressions of other proteins causing proton leak, such as SOD, catalase, and ANT, should be considered for future study. SOD and catalase protein expressions are affected by oxidative stress, which is ultimately caused by acylcarnitines, and as an uncoupling protein, ANT expressions should be studied as well.

References

Koves, *et al*. (2007). *Acylcarnitine*, C735.
Mailloux and Harper. (2011). *Electron transport chain*, 454.

Acknowledgements

I would like to thank UROP for this great research opportunity. I would also like to thank Dr. Brianne Thrush for her guidance throughout the semester, Dr. Mary-Ellen Harper for overseeing this project, and everyone else in the Harper Lab for making this an exceptional experience.