Quantification of steroids in tissues between Raldh1-KO and WT mice

Michael Cockrum 1,2; Hong Sik Yoo 2; Joseph L. Napoli 2
1SPUR Fellow, 2Department of Nutritional Science and Toxicology

Abstract

Vitamin A has many roles throughout the body including vision, cell differentiation, immune function, and intermediary metabolism. Focus of vitamin A research was given primarily to all-trans retinoic acid (atRA), a carboxylated form of vitamin A since atRα was found to be associated with important transcription factors whereas retinal and retinol were thought to be only used as precursors for atRA. However, a research study showed that Raldh1-KO mice resist diet-induced obesity, which triggered investigations on the role of retinaldehyde (retinal) in maintaining energy balance. Originally, the increased brown adipose tissue (BAT) activity was suggested as an underlying mechanism, which has not been reproduced elsewhere. To find a possible link, the Napoli lab carried out an RNA sequencing of Raldh1-KO mice and found higher expression of Sult1e1, Sult2a1, Sult2a2, and Sult2a5 which are involved in the sulfation of E2, DHEA, and bile acids.

Hypothesis

To find an unknown linkage between ablation of Raldh1 and energy balance, the Napoli lab conducted an mRNA sequencing comparing KO and WT liver. This showed higher expression of Sult1e1, Sult2a1, Sult2a2, and Sult2a5 which are involved in the sulfation of E2, DHEA, and bile acids.

Materials and Methods

Breeding scheme:

Raldh1 heterozygote KO mice, backcrossed for 5 generations, were used to produce homogyote KO and WT mice.

Comparison of Body Weights

Female WT and KO mice were used. Tissues should be collected at 10 weeks instead of 7-8 weeks to allow steroid concentrations to increase and improve quantification.

Future direction should focus on quantifying steroid levels in the collected liver tissues as this is where SULT enzymes are differentially expressed.

• A higher sample size should be used in future experiments to improve accuracy of the results. Due to difficulties with breeding and time constraints of the project only 3 male WT and 4 female WT mice were used. Tissues should be collected at 10 weeks instead of 7-8 weeks to allow steroid concentrations to increase and improve quantification.

• When collecting tissue two male KO mice had rough liver morphology. These two male KO mice also had yellow serum which may be indicative of high bilirubin and/or liver damage. Further experimentation should be done to determine if this is reproducible since the Raldh1-KO has previously been reported as a healthy phenotype.

References


Conclusion and Future Direction

- T and DHEA sulfates were detected in serum. There was no significant difference in Free T concentrations between genotypes. No significant difference between genotypes was observed in sulfated DHEA.
- Both male and female WT mice appear to have gained more weight than KO mice, however this difference is not significantly different. The low sample size, tissue collection date, and LFD used are all contributing factors.
- Due to COVID-19 the project was not able to be completed. Future direction should focus on quantifying steroid levels in the collected liver tissues as this is where SULT enzymes are differentially expressed.

Acknowledgements

Thanks to Hong Sik Yoo for all the guidance and mentorship for the past two semesters. Thank you to Dr. Joseph Napoli for supporting the project. Thanks to Yaxin Zhao and Nina Fox for assistance in tissue collection. Thanks to the CNR SPUR program for this opportunity.