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

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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 atRA was found to be associated with important transcription factors whereas retinol and retinal 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 retinyl aldehyde (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 liver and found higher expression of Sult1e1, Sult2a1, Sult2a2, Sult2a5, all of which are involved in steroid sulfation compared to WT mice. From these findings, I hypothesize that the ratio of sulfated vs free steroids is higher in the liver of Raldh1-KO mice compared to WT. Liver tissues, serum, eWAT, iWAT, and BAT from WT and Raldh1-KO will be collected, and an HPLC-MS/MS assay will be done for quantification of free and sulfated steroid levels. This research is unique in that I seek the cause of metabolic imbalance in the intersection between vitamin A and steroid metabolism.

Background

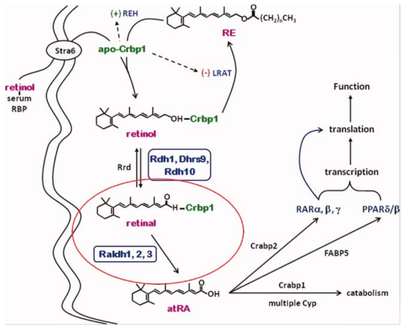
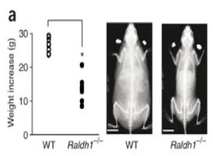


Figure 1. Vitamin A metabolism.Raldh1 catalyzes the oxidation of retinal to retinoic acid



Raldh1 ablation results in a phenotype that resists diet induced obesity. It has been hypothesized that the observed phenotype is due to increased BAT activity

Figure 2. Raldh1 ablated mice Mice fed a HFD resist diet induced obesity after 180 days [3].

Further investigation by the Napoli lab into the Raldh1 phenotype has shown that retinal concentrations do not increase in sufficient amounts to support the activation these nuclear receptors. In addition, atRA tissue concentrations did not differ, except in liver which had a decrease [4].

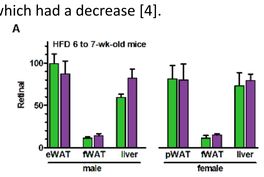


Figure 3. Mice fed a HFD did not have increased retinal concentrations, except the liver which was not sufficient to act on the proposed nuclear receptors [4].

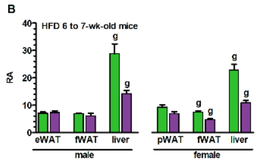


Figure 4. atRA in ko mice did not differ, except in liver which had decreased concentrations [4].

Background

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.

Gene	Fold change	Function
Sult2a6	112	Sulfates DHEA (and bile acids)
Sult1e1	13	Sulfates estrogen
Sult2a1	603	Sulfates DHEA (and bile acids)
Sult2a2	179	Sulfates DHEA (and bile acids)
Sult2a5	Very High	Sulfates DHEA (and bile acids)

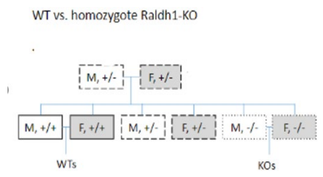
Figure 5. mRNA sequencing of WT and KO mice fed a LFD. Tissus collected at 7-8 weeks, fasted for 16 hours. Increased expression of SULT enzymes in Raldh1-KO mice

Hypothesis

The ratio of sulfated vs free steroids is higher in the liver of Raldh1-KO mice compared to WT.

Materials and Methods

Breeding scheme:



Raldh1 heterozygote KO mice, backcrossed for 5 generations, were mated to produce homozygote KO and WT mice.

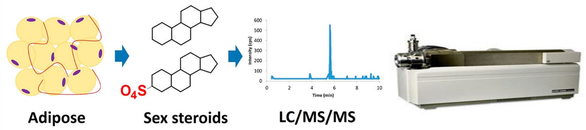
Figure 4. Mouse breeding scheme 5 pairs from backcross for 5 generations

Tissue collection:

Serum, liver, eWAT, iWAT, and BAT were collected from 7-8 weeks old male and female mice, fed a 4IU vitamin A diet, after fasting for 16 hours.

Quantification of steroids using LC/MS/MS:

For samples with concentrations below the lower limit of detection (LLOD) the highest concentration below the LLOD was used [6]. This was done to prevent distortion of relevant data due to missing data points. The API 4000 LC/MS/MS System was used for tissue steroid quantification.



Results

Serum Steroid Quantification

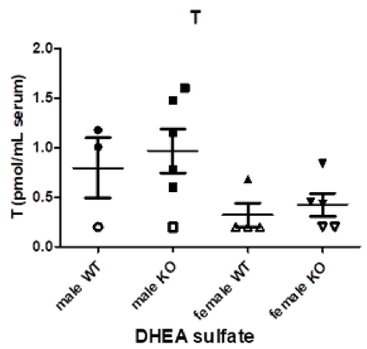


Figure 6. Blood serum concentrations of testosterone (T) do not differ between KO and WT of both male and female. For samples with concentrations below the lower limit of detection (LLOD) the highest concentration below the LLOD was used. These samples are represented using an open shape.

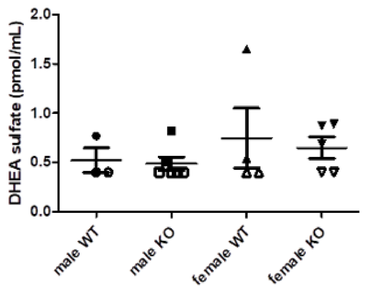


Figure 7. Blood serum concentrations of sulfated Dehydroepiandrosterone(DHEA) do not differ between KO and WT of both male and female. For samples with concentrations below the lower limit of detection (LLOD) the highest concentration below the LLOD was used. These samples are represented using an open shape.

Comparison of Body Weights

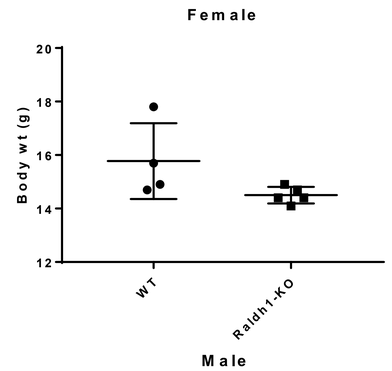


Figure 8. Body weights were not statistically different between Female WT and KO mice. (Mean± SEM, 15.78 ± 0.7087, n=4 for WT, 14.5 ± 0.1378, n=5 for KO. Two- tailed P-value=0.0873)

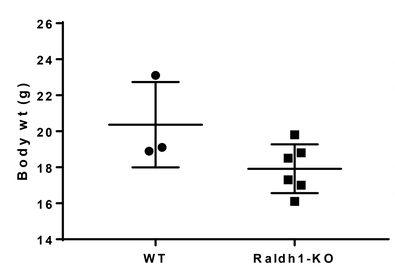


Figure 9. Body weights were not statistically different between Male WT and KO(Mean±SEM 20.37 ± 1.368, n=3 for WT, 17.92 ± 0.5534, n=6 KO. Two- tailed P- value= 0.0820)

Conclusion and Future Direction

- T and DHEA sulfate 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.
- A higher sample size should be used in future experiments to improve accuracy of the results. Due to difficulty 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

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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.

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