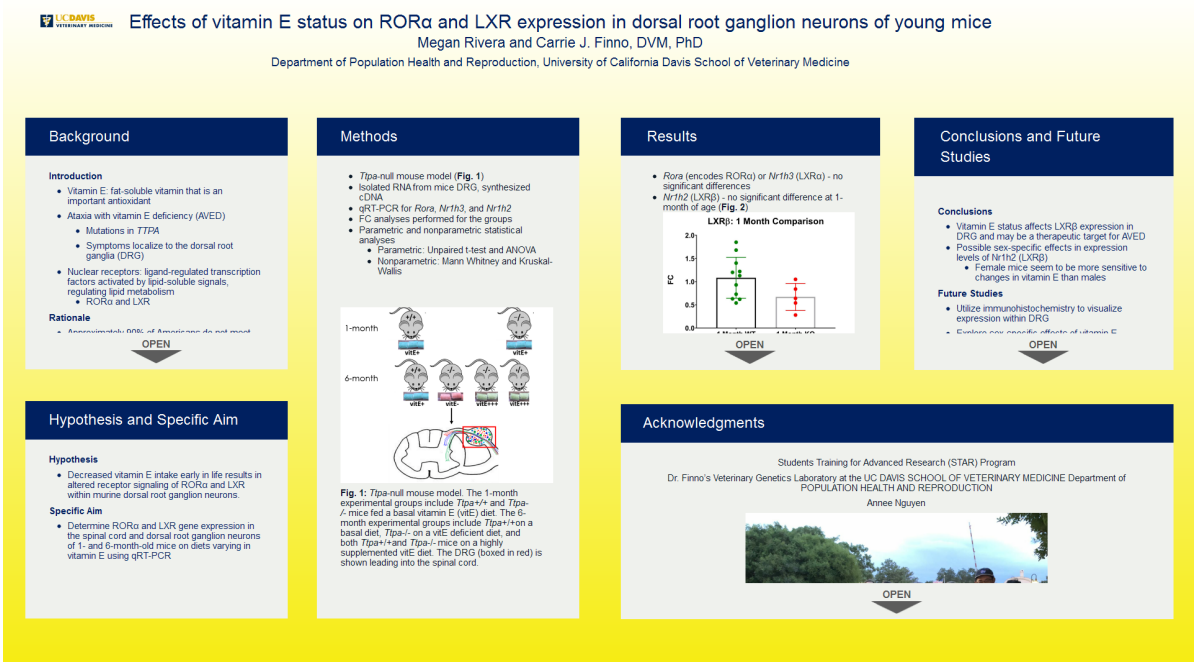
Effects of vitamin E status on RORα and LXR expression in dorsal root ganglion neurons of young mice



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BACKGROUND

Introduction

- Vitamin E: fat-soluble vitamin that is an important antioxidant
- Ataxia with vitamin E deficiency (AVED)
  - Mutations in *TTPA*
  - Symptoms localize to the dorsal root ganglia (DRG)
- Nuclear receptors: ligand-regulated transcription factors activated by lipid-soluble signals, regulating lipid metabolism
  - RORα and LXR

Rationale

- Approximately 90% of Americans do not meet the recommended intake requirements for vitamin E (1)
  - Subclinical effects?
- Previous studies - cerebrum and cerebellum and include individuals that already show clinical signs
  - Molecular dysregulation begins in spinal cord (2)
  - 1- and 6-month-old mice
  - Sex effects have not been evaluated (3)

HYPOTHESIS AND SPECIFIC AIM

Hypothesis

- Decreased vitamin E intake early in life results in altered receptor signaling of RORα and LXR within murine dorsal root ganglion neurons.

Specific Aim

- Determine RORα and LXR gene expression in the spinal cord and dorsal root ganglion neurons of 1- and 6-month-old mice on diets varying in vitamin E using qRT-PCR

METHODS

- *Ttpa*-null mouse model (**Fig. 1**)
- Isolated RNA from mice DRG, synthesized cDNA
- qRT-PCR for *Rora*, *Nr1h3*, and *Nr1h2*
- FC analyses performed for the groups
- Parametric and nonparametric statistical analyses
  - Parametric: Unpaired t-test and ANOVA
  - Nonparametric: Mann Whitney and Kruskal-Wallis

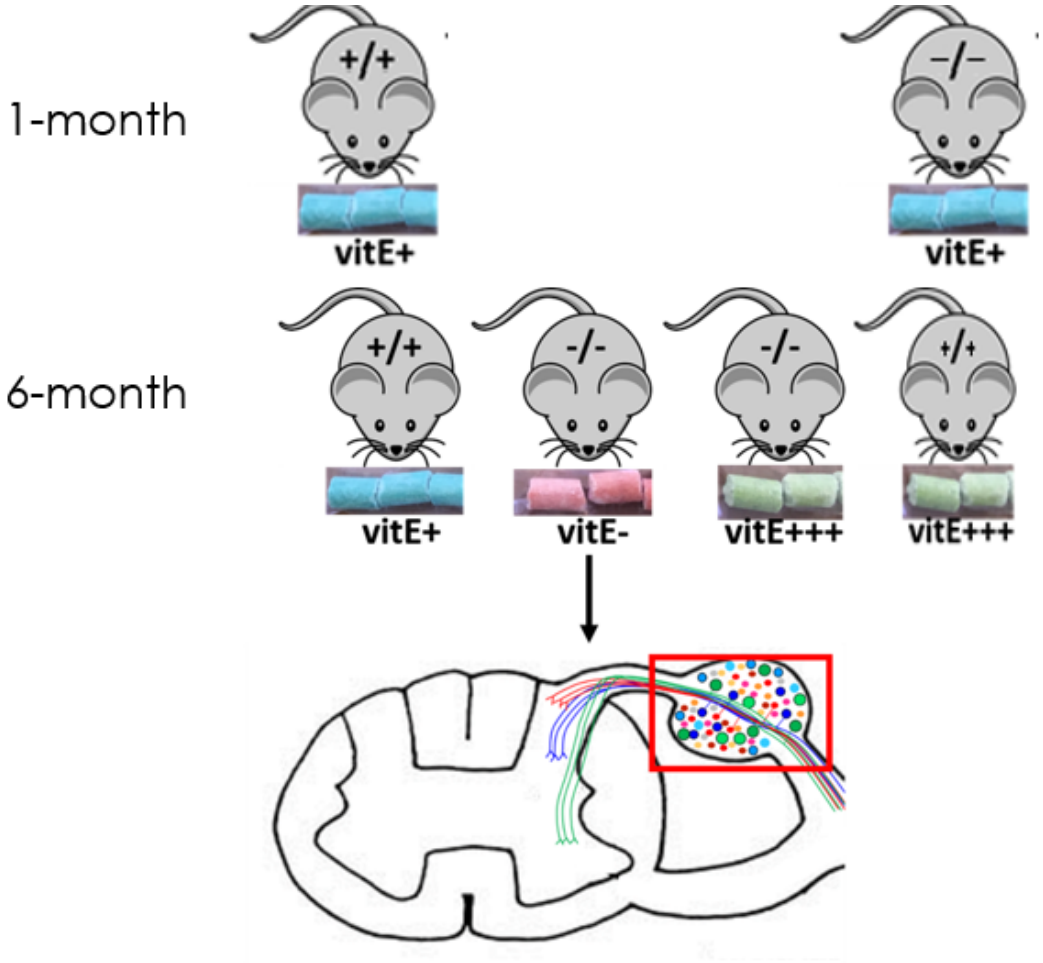

Fig. 1: *Ttpa*-null mouse model. The 1-month experimental groups include *Ttpa*+/+ and *Ttpa*-/- mice fed a basal vitamin E (vitE) diet. The 6-month experimental groups include *Ttpa*+/+on a basal diet, *Ttpa*-/- on a vitE deficient diet, and both *Ttpa*+/+and *Ttpa*-/- mice on a highly supplemented vitE diet. The DRG (boxed in red) is shown leading into the spinal cord.

RESULTS

- *Rora* (encodes RORα) or *Nr1h3* (LXRα) - no significant differences
- *Nr1h2* (LXRβ) - no significant difference at 1-month of age (**Fig. 2**)

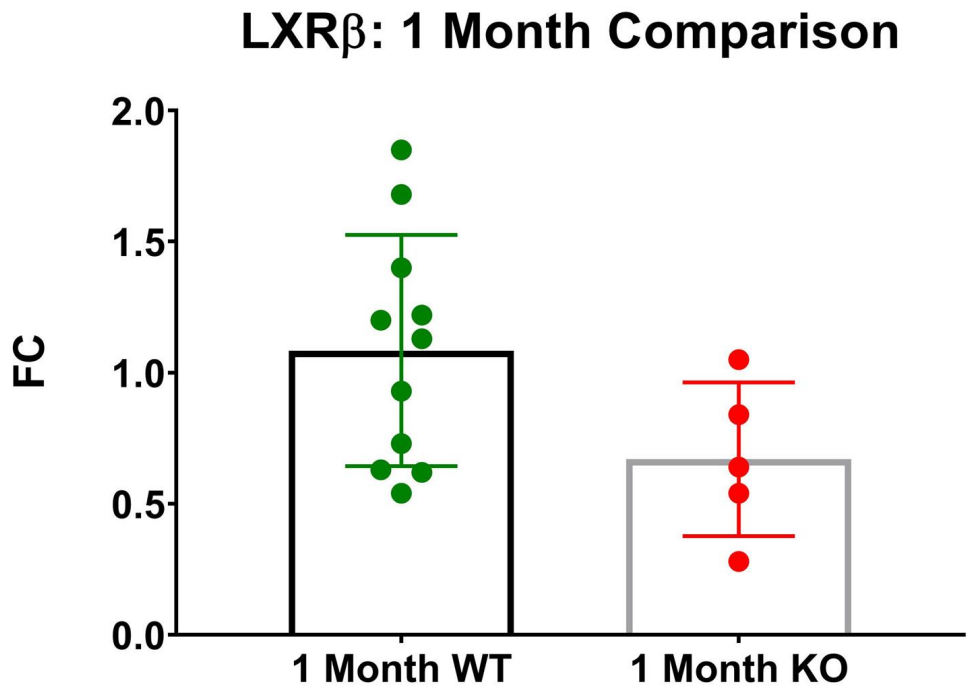

Fig. 2: Comparison of fold change values for LXRβ in 1-month mice.

- *Nr1h2* (LXRβ) - significant difference between 6-month-old mice (**Fig. 3)**
  - $Ttpa^{+/+}$ supplemented and $Ttpa^{-/-}$ deficient (p=0.006)

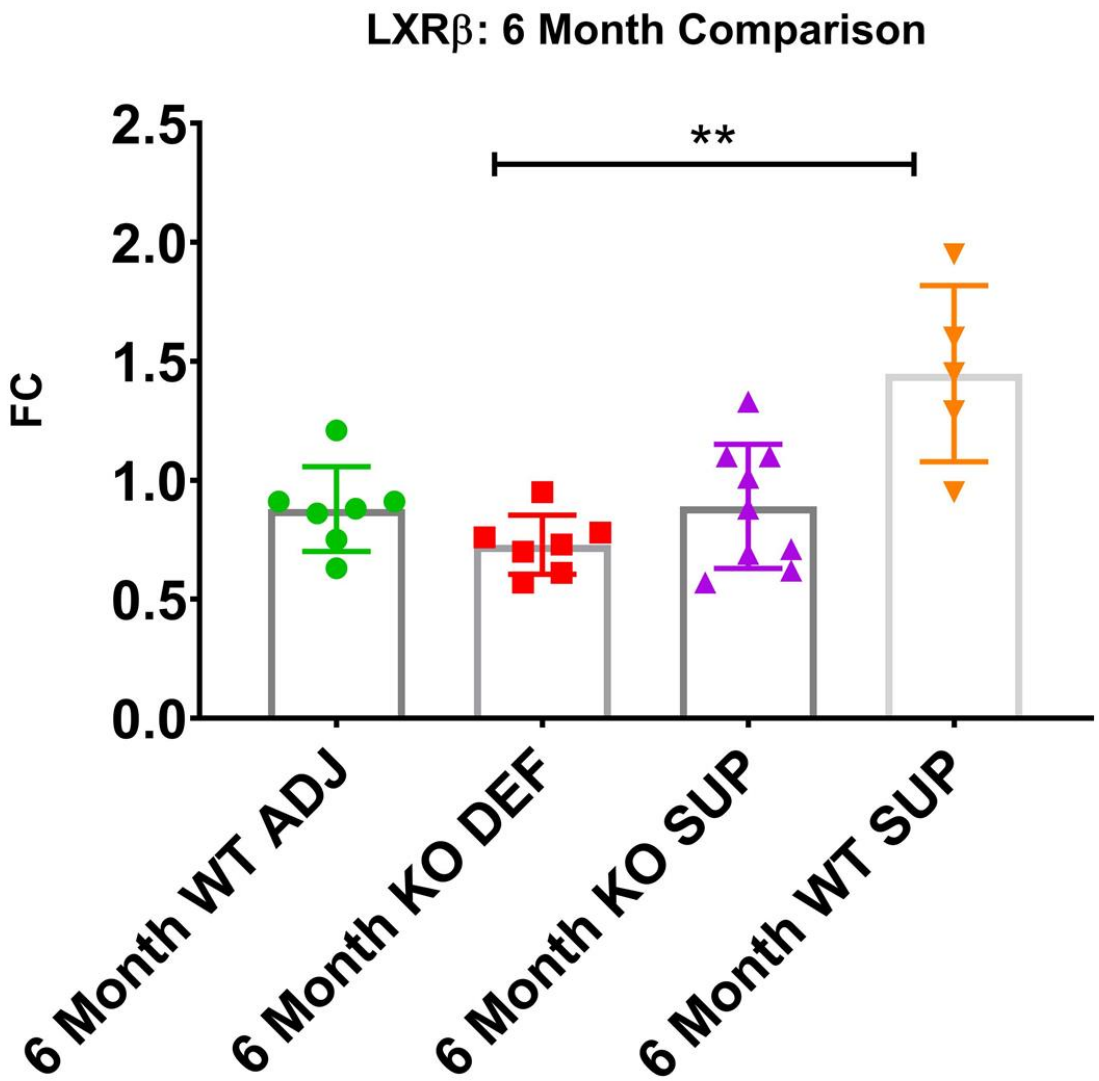

Fig. 3: Comparison of fold change values for using LXRβ in 6-month mice . **p<0.01

- *Nr1h2* (LXRβ) - no significant difference at 1-month of age (**Fig. 4**)

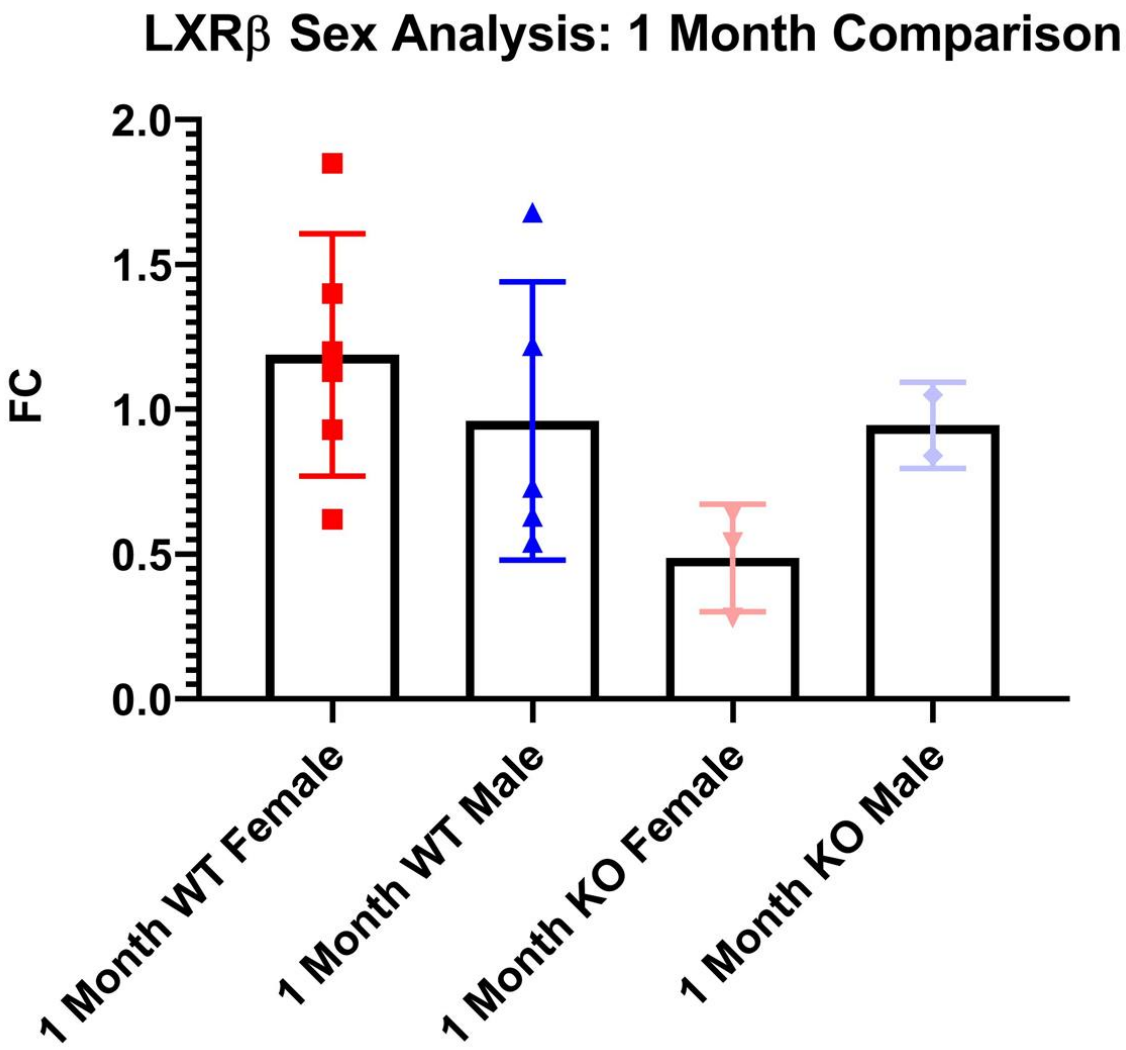

Fig. 4: Sex-specific comparison of fold change values for LXRβ in 1-month mice. 1-month WT (both sexes) was used to generate the baseline comparison group for fold change calculations.

- *Nr1h2* (LXRβ) - significant difference between 6-month-old mice in sex-specific expression **(Fig. 5)**.
  - $Ttpa^{-/-}$ deficient male and $Ttpa^{+/+}$ supplemented female (p=0.026)

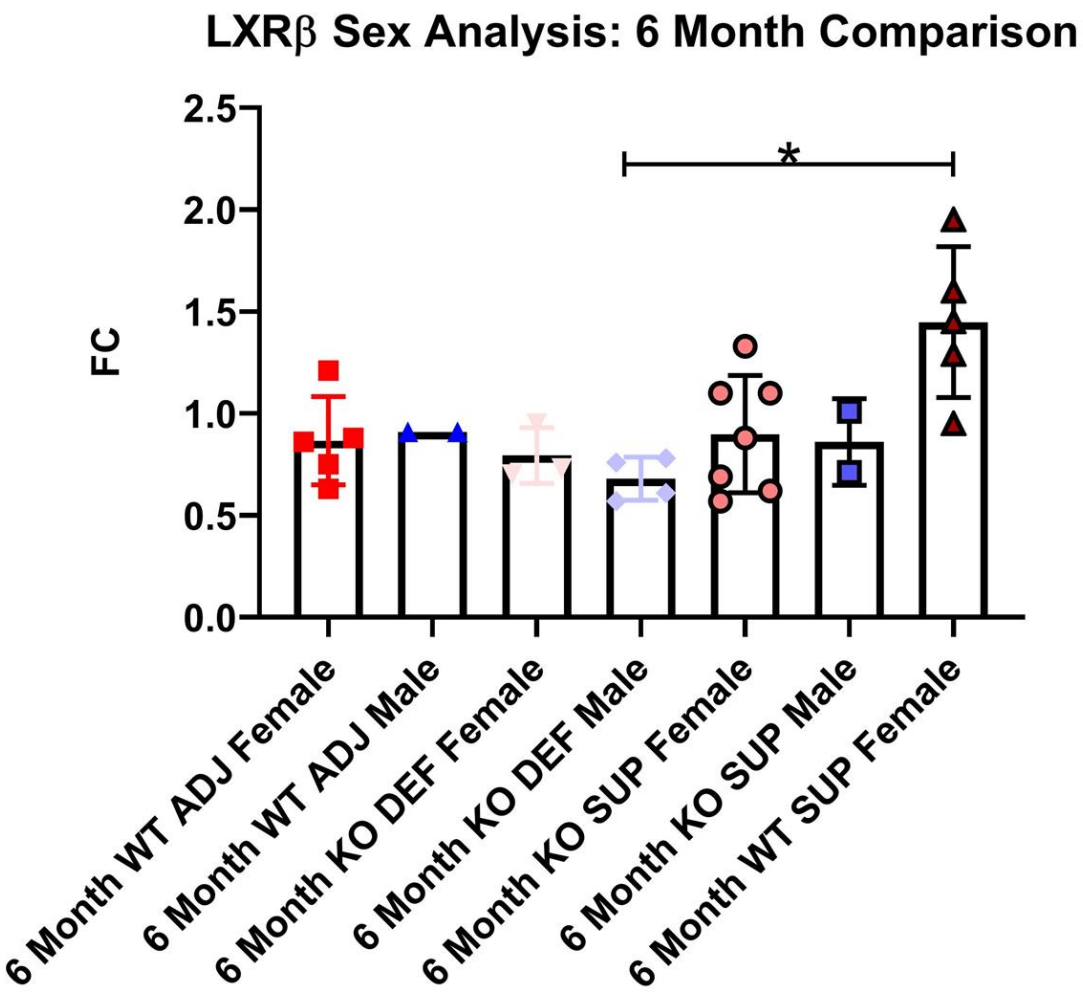

Fig. 5: Sex-specific comparison of fold change values for LXRβ in 1-month mice. 1-month WT (both sexes) was used to generate the baseline comparison group for fold change calculations. *p<0.05.

CONCLUSIONS AND FUTURE STUDIES

Conclusions

- Vitamin E status affects LXRβ expression in DRG and may be a therapeutic target for AVED
- Possible sex-specific effects in expression levels of Nr1h2 (LXRβ)
  - Female mice seem to be more sensitive to changes in vitamin E than males

Future Studies

- Utilize immunohistochemistry to visualize expression within DRG
- Explore sex-specific effects of vitamin E
  - Small n impacted power of analysis
  - Females may be more sensitive to changes in vitamin E than males

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ABSTRACT

Approximately ninety percent of Americans do not meet the recommended intake requirements for vitamin E (vitE). Severe deficiency associated with mutations in tocopherol transfer protein alpha (TTPA), affecting vitE transport, leads to ataxia with vitE deficiency (AVED). AVED localizes to the dorsal root ganglia (DRG), with symptoms developing during childhood. RORα and LXR are important nuclear receptors linked to vitE. Nuclear receptors are ligand-regulated transcription factors that are activated by lipid-soluble signals, regulating lipid metabolism. RORα and LXR were hypothesized to be alternatively expressed in the DRG depending on vitE status. We explored the effects of vitE status on the age-dependent expression of RORα and LXR in DRG neurons using the *Ttpa*-null mouse model. Six experimental groups were evaluated via qRT-PCR; *Ttpa*+/+ and *Ttpa*-/- at 1 mo. of age on a basal diet and 6 mo. *Ttpa*+/+on a basal diet, 6 mo. Ttpa-/- on a vitE deficient diet and both 6 mo. Ttpa+/+and Ttpa-/- mice on a highly supplemented vitE diet. There were no differences in expression levels of *Rora* (encodes RORα) or *Nr1h3* (LXRα) across experimental groups. There was no effect of age on *Nr1h2* (LXRβ) within genotypes. However, Ttpa+/+mice maintained on a highly supplemented vitE diet had significantly higher DRG expression of *Nr1h2* than Ttpa-/- on a vitE deficient diet (p=0.006). Therefore, vitE status affects LXRβ expression in DRG neurons and may be a therapeutic target for AVED.

REFERENCES

1. Fulgoni, V. L., Keast, D. R., Bailey, R. L., & Dwyer, J. (2011). Foods, fortificants, and supplements: Where do Americans get their nutrients?. The Journal of Nutrition, 141 (10), 1847-1854 .https://doi.org/10.3945/jn.111.142257
2. Finno, C. J., Bordbari, M. H., Gianino, G., Ming-Whitfield, B., Burns, E., Merkel, J., … Miller, A. D. (2018). An innate immune response and altered nuclear receptor activation defines the spinal cord transcriptome during alpha-tocopherol deficiency in Ttpa-null mice. Free Radical Biology and Medicine, 120, 289-302.
3. Finno, C. J, Bordbari, M. H., Valberg, S. J., Lee, D., Herron, J. Hines, K., … Xu, L. (2016). Transcriptome profiling of equine vitamin E deficient neuroaxonal dystrophy identifies upregulation of liver X receptor target genes. Free Radical Biology and Medicine, 101, 261-271. https://doi.org/10.1016/j.freeradbiomed.2016.10.009