ALTERATIONS IN NEURAL FATTY ACID METABOLISM CAUSED BY VITAMIN E DEFICIENCY

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Outline

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- Hypothesis
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- Model organism
- Methods
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- Questions

Significance

- Oxidative damage in the brain is believed to play a role in degenerative diseases such as Alzheimer's and dementia
- Patients with Alzheimer's have low levels of vitamin E in their cerebrospinal fluid (Kontush et al., Acad Science 2004)
- Alpha tocopherol supplementation of 2000 IU has been shown to slow the progression of Alzheimer's and dementia, but the mechanism is unknown. (Sano M, Ernesto C, Thomas RG, et al, N Engle J Med. 1997.)

Vitamin E (Alpha tocopherol)

Exists in 8 isomers:

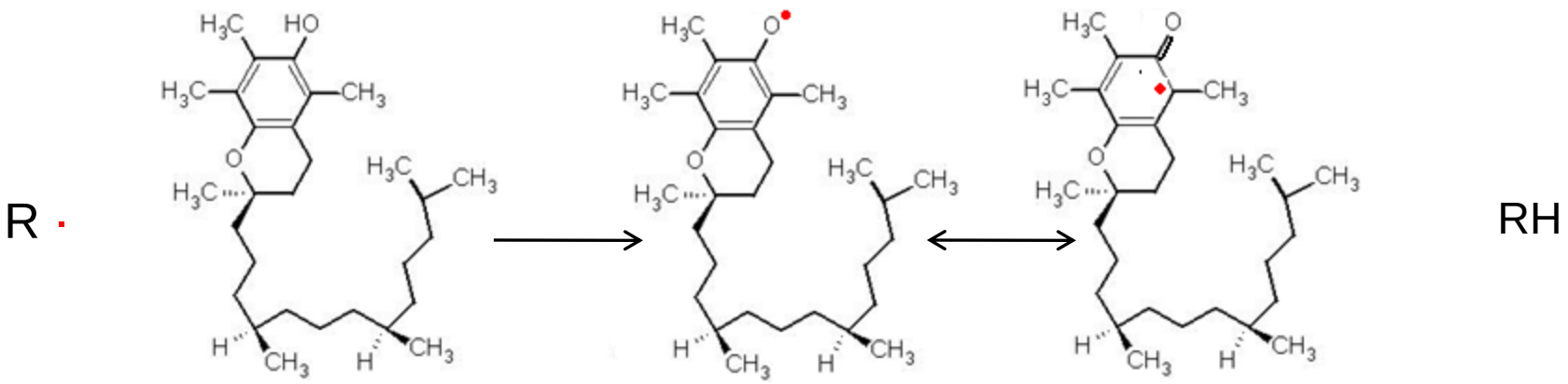
- Alpha, beta, gamma, and delta tocopherol
- Alpha, beta, gamma, and delta tocotrienol
- Alpha tocopherol is preferentially retained due to the action of the tocopherol transfer protein in the liver (DRI, 2000)

Vitamin E (Alpha tocopherol)

- Lipid soluble vitamin
- Principal role is to protect polyunsaturated fatty acids from peroxidation
- Deficiency can cause peripheral neuropathy

Vitamin E (Alpha tocopherol)

- Donates a hydrogen from the free hydroxl group on the aromatic ring



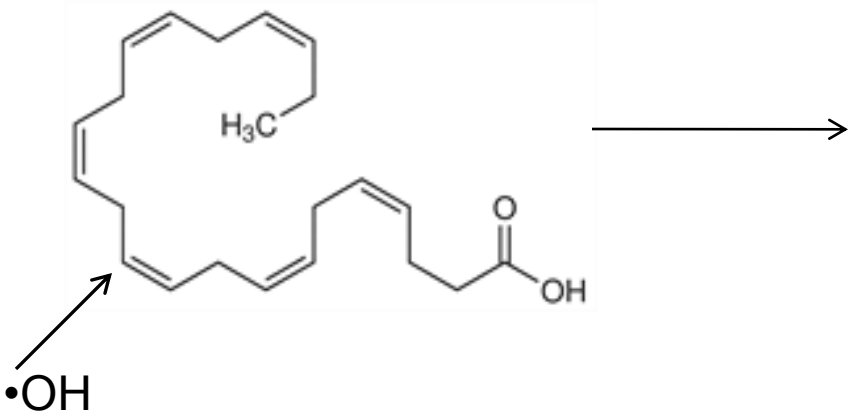
DHA (Docosahexaeonic acid) (22:6 ω-3)

- Important long chain fatty acid created by elongating omega 3 fatty acids or consumed as fish oil
- Comprises ~50% of brain fatty acids

  (Bazar, et al. AnnuRev Nutr 2011)
- Wide variety of cellular functions:
  - Gene regulation, membrane fluidity, precursor for some signaling molecules

DHA

- DHA is very susceptible to peroxidation due to the high amount of unsaturation in the aliphatic tail



Hypothesis

We hypothesize that vitamin E protects DHA from lipid peroxidation

Prediction

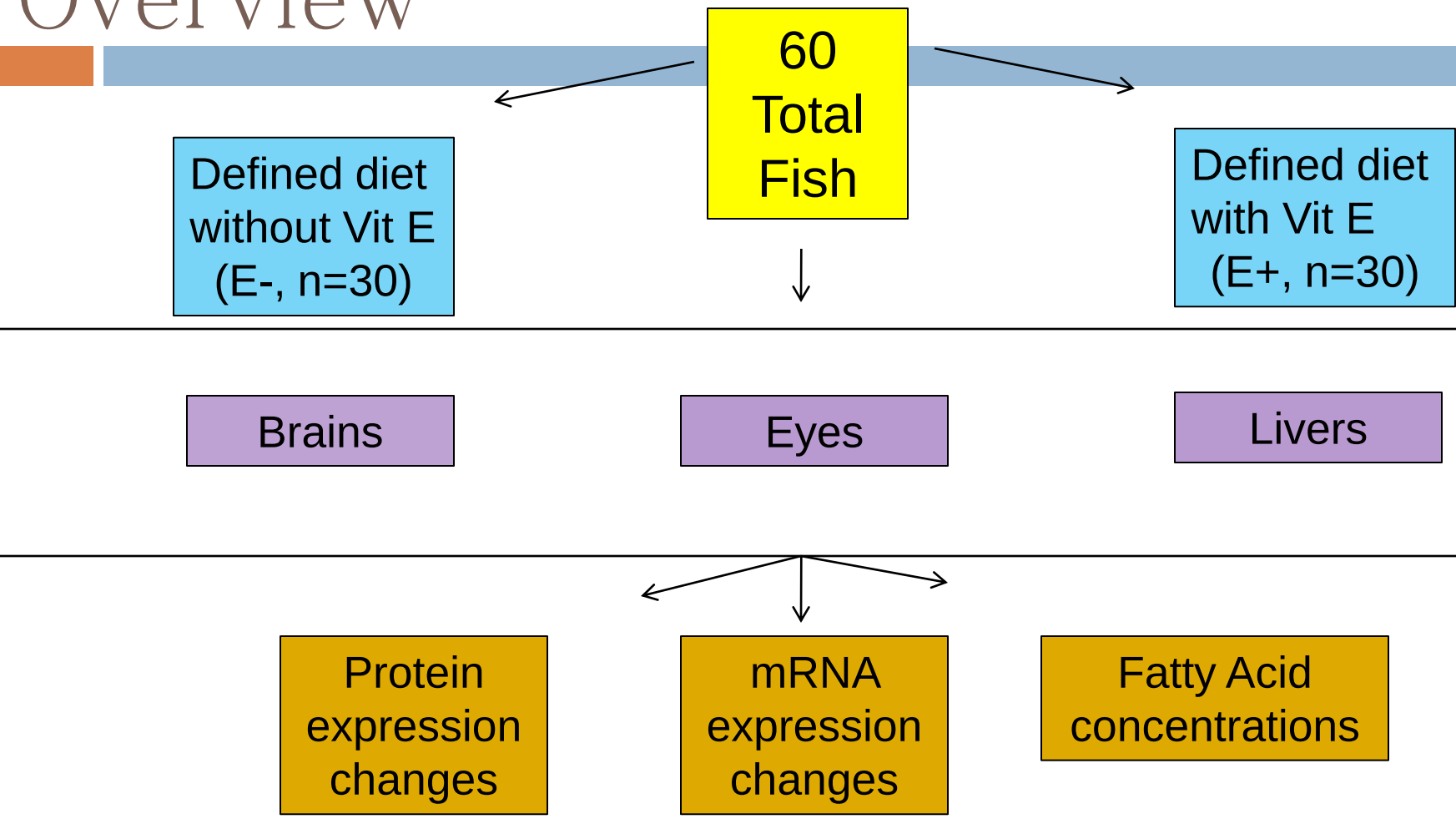
Zebrafish fed a vitamin E deficient diet:

1) Will have increased levels of lipid peroxidation
2) Will have an increase in mRNA expression of enzymes responsible for polyunsaturated fatty acid synthesis to replace any DHA lost to lipid peroxidation
3) However, due to the deficiency of vitamin E in the diet, the increase in mRNA expression will be insufficient to replace DHA lost in the brain.

Model Organism
Zebrafish

- Currently established model in the Traber/Tanguay laboratories
- Similar fatty acid metabolism pathways as humans (Lebold et al J Nutr, accepted)
- Easily manipulated diet with defined ingredients

Method Overview



Proteins involved in fatty acid synthesis

1. Sterol regulatory binding factor 1 (SREBP1)
2. Fatty acid desaturase (fads2)
3. Elongase (elovl2)
4. Elongase (elovl4)

Sterol regulatory binding factor 1 (*SREBP1*)

- Regulates genes related to lipid metabolism and fatty acid synthesis

Fatty acid desaturase (*fads2*)

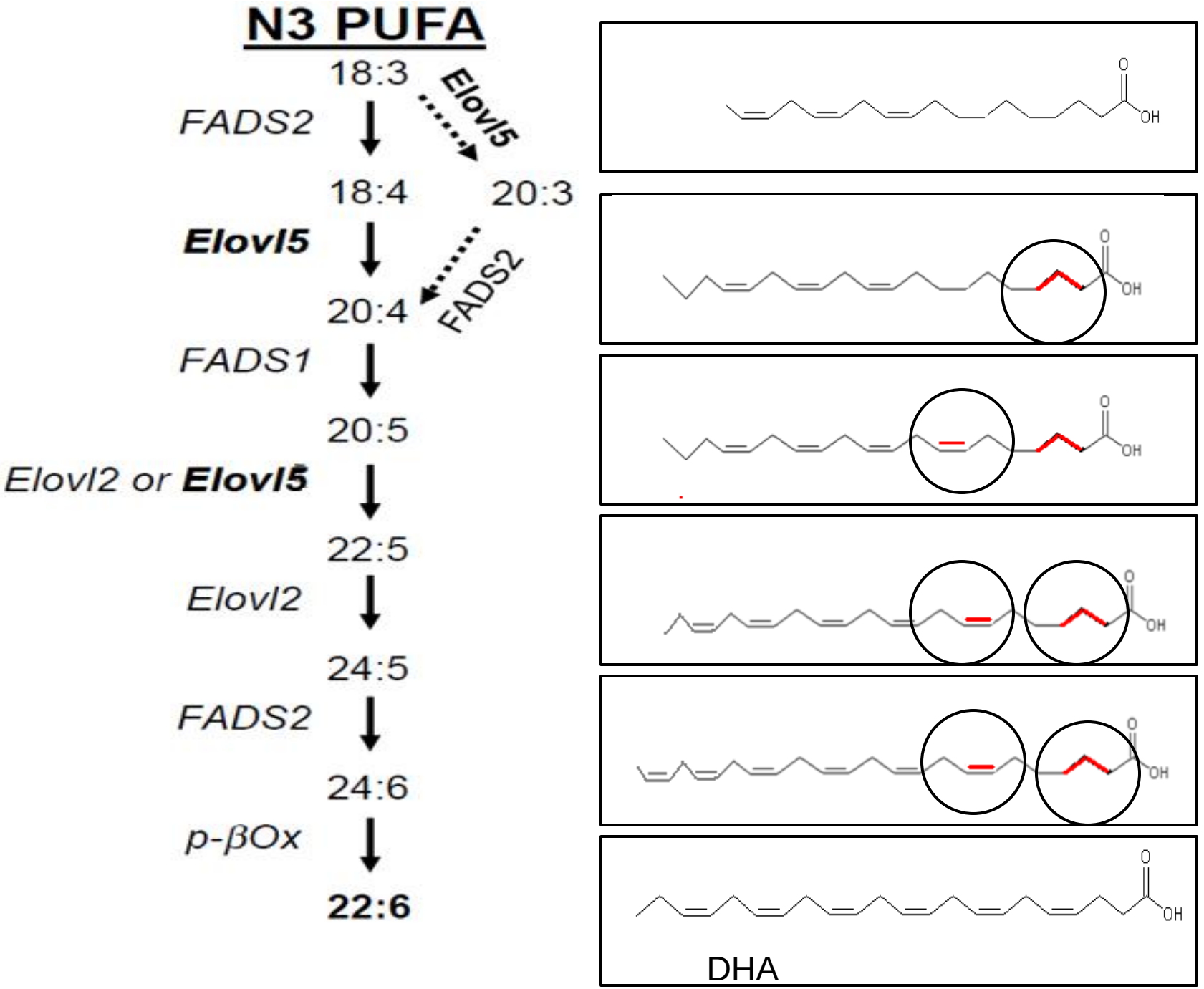
- Removes two hydrogens from a fatty acid in order to create a double bond
- Required for the synthesis of DHA from Omega-3 fatty acids
- mRNA measured in the liver and brain

Elongase (*elovl2*)

- Catalyzes the synthesis of polyunsaturated very long chain fatty acids
- Elongates fatty acids by adding 2 carbons
- mRNA measured in the liver and brain

Elongase (*elovl4*)

- Catalyzes the synthesis of polyunsaturated very long chain fatty acids
- Elongates fatty acids by adding 2 carbons
- Specifically expressed in neural tissues
- mRNA measured in the eye and brain



Tripathy S, Torres-Gonzalez M, Jump DB. J Lipid Res. 2010 Sep;51(9):2642-54

Methods

- Adult zebrafish fed a vitamin E sufficient or deficient diet were euthanized and brains, livers, and eyes removed for analysis

Methods

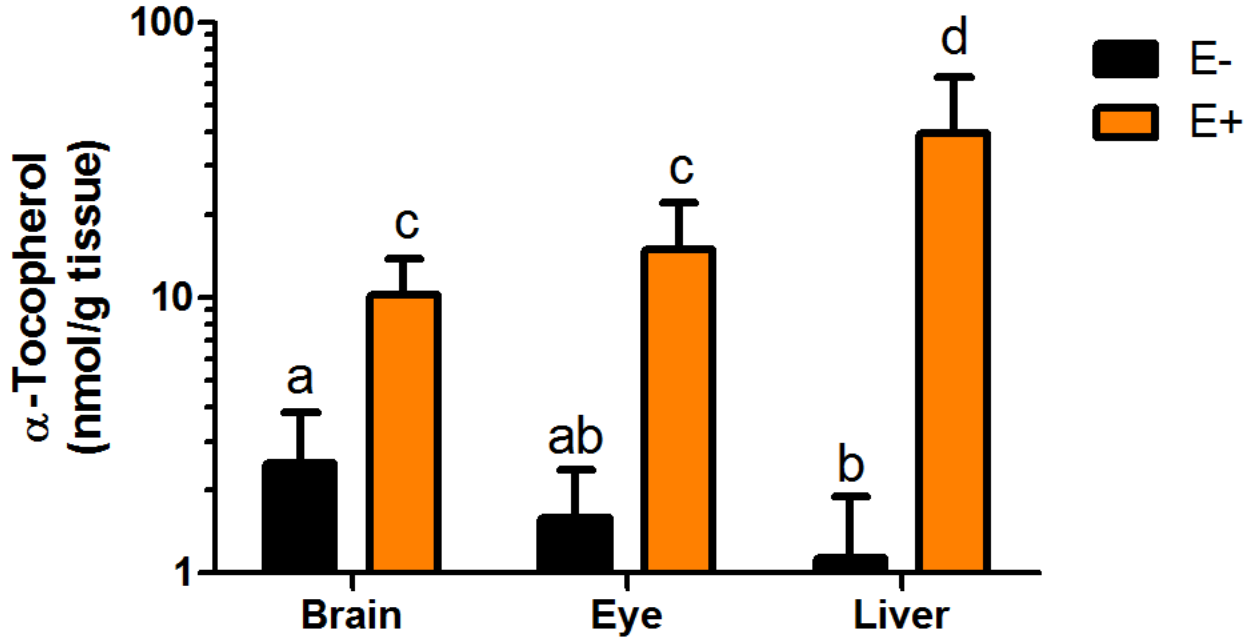
- Fatty acid concentrations were determined using high pressure liquid chromatography coupled to a single-quad mass spectrometer
- Vitamin E concentrations were determined using high pressure liquid chromatography with electrochemical detection

Methods

- mRNA expression, evaluated using quantitative real time PCR, were:
  - fatty acid desaturase (fasd2)
  - elongase (elovl2, elovl4)
- Protein expression of SREBP1 was determined using western blotting

Results

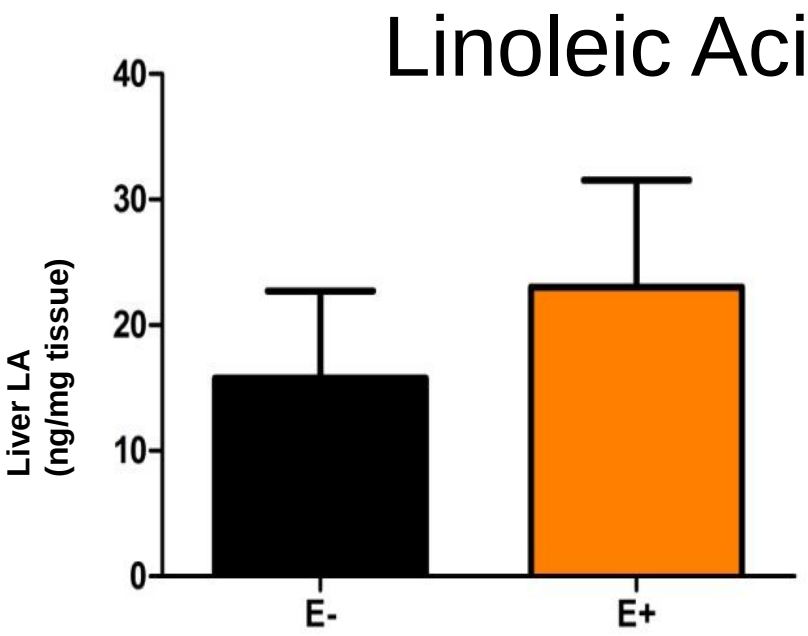
Diet impact on vitamin E levels

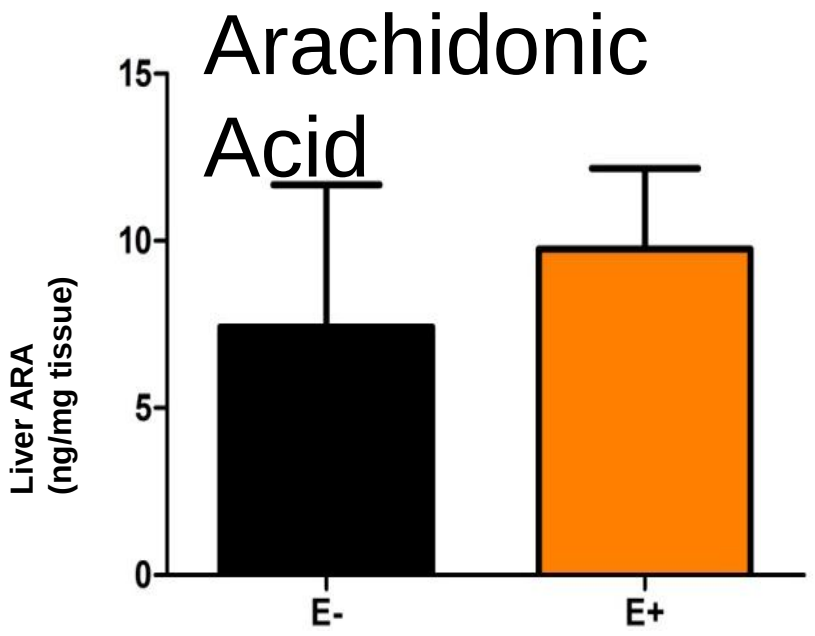


Diet	Tissue	Diet x Tissue
P<0.0001	P=0.2772	P=0.0001

Columns not sharing the same letter are significantly different

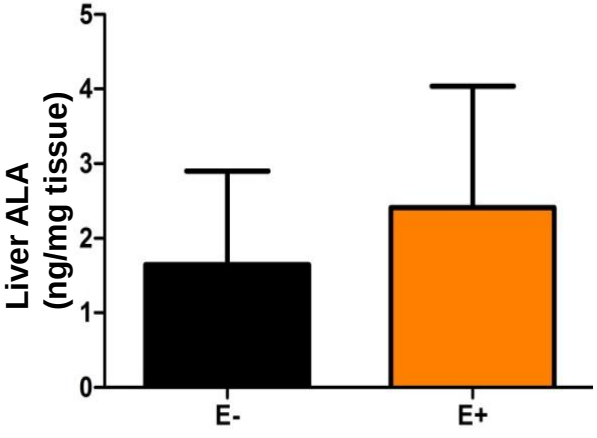
Vitamin E deficiency did not affect fatty acid concentrations in the liver



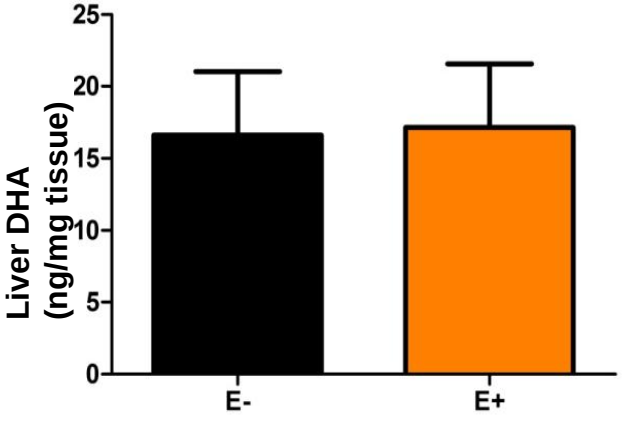


Vitamin E deficiency did not affect fatty acid concentrations in the liver

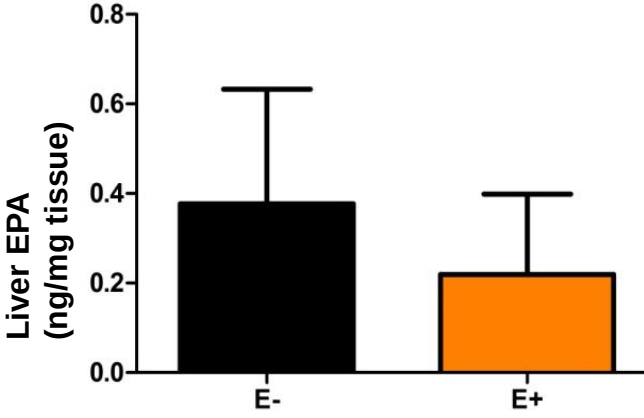
Alpha Linolenic Acid



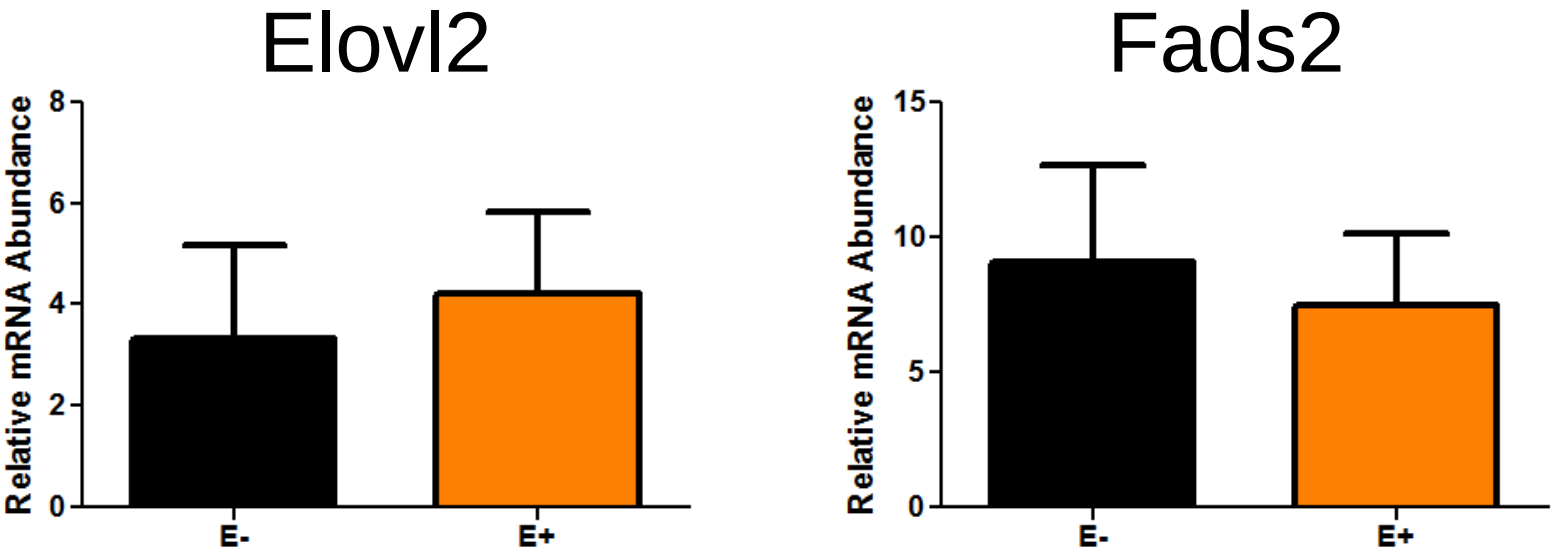
DHA



EPA



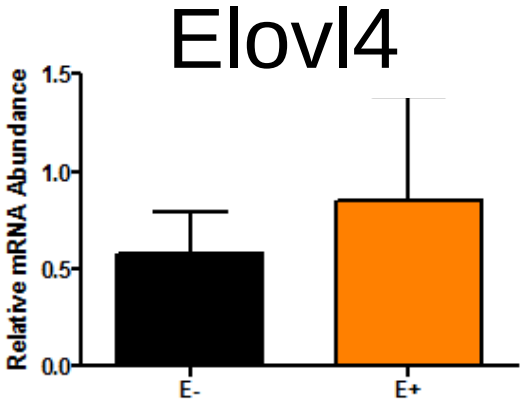
Vitamin E deficiency did not affect Elovl2 or FADs2 mRNA expression in the liver

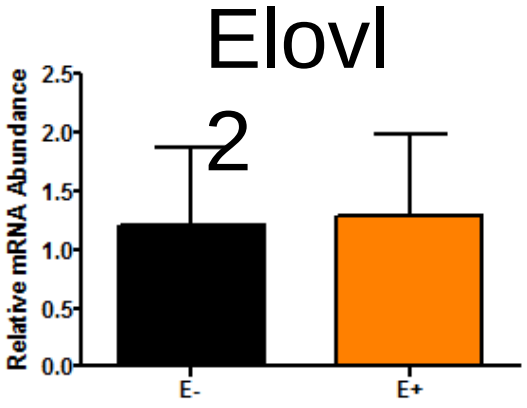


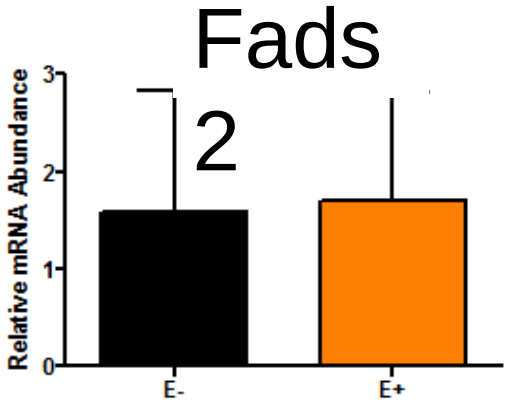
Fatty Acid Concentration in the Brain

- No data available due to equipment failure.

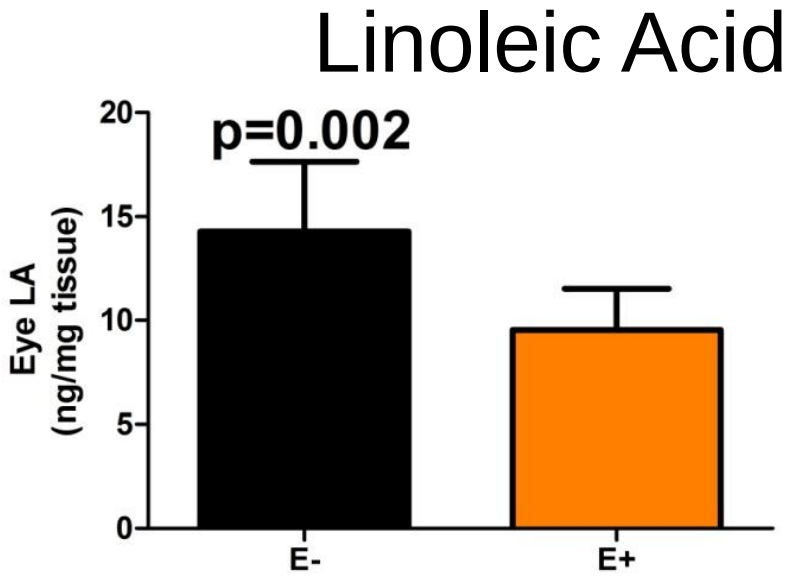
Vitamin E deficiency did not affect mRNA expression in the brain

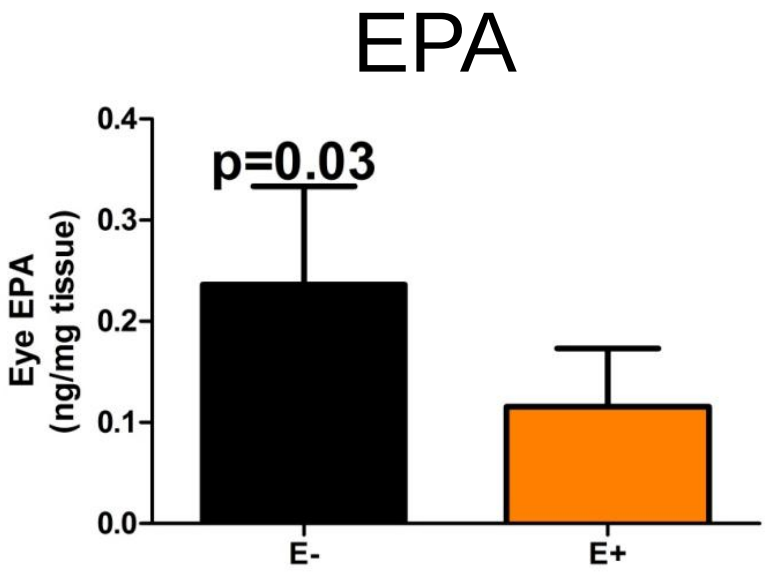






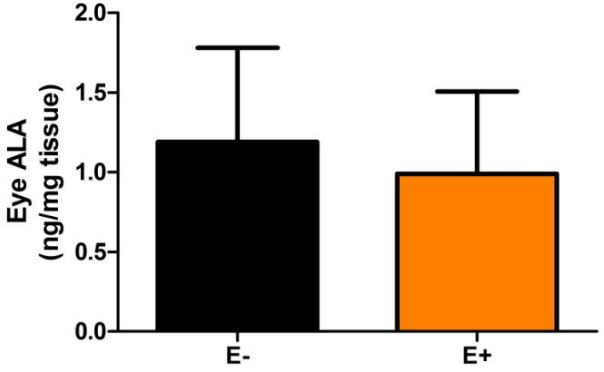
Vitamin E deficiency increases eye EPA and linoleic acid concentrations



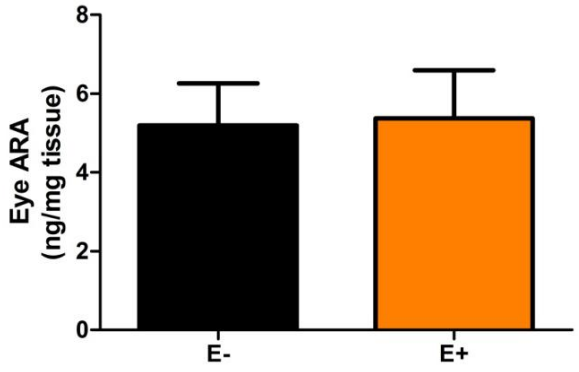


Vitamin E deficiency tended to decrease DHA concentration in the eye

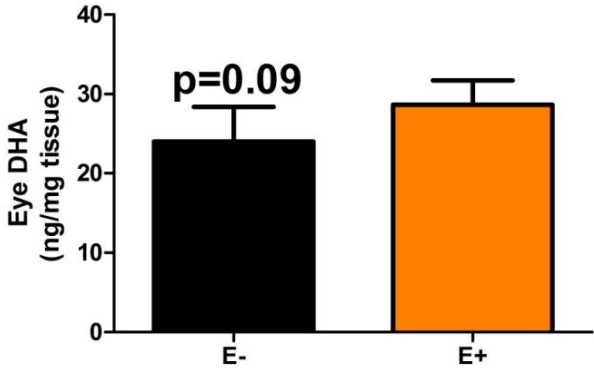
Alpha Linolenic Acid



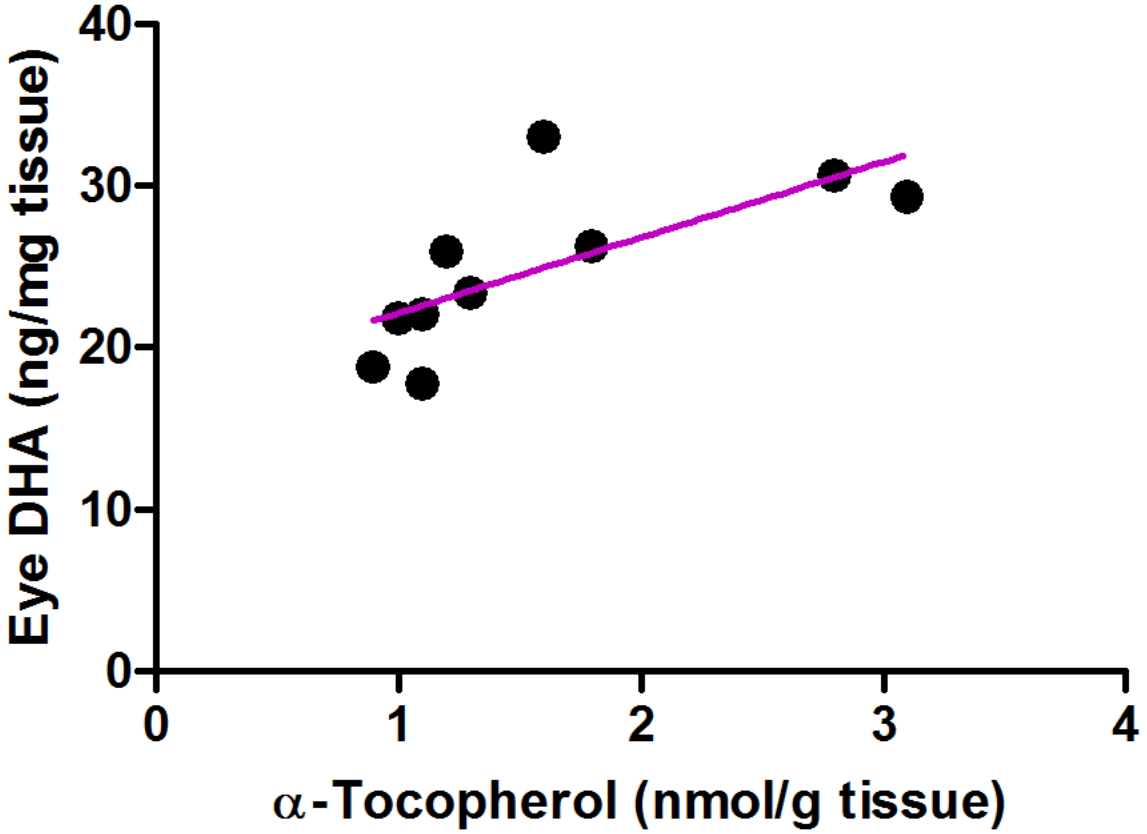
Arachidonic Acid



DHA

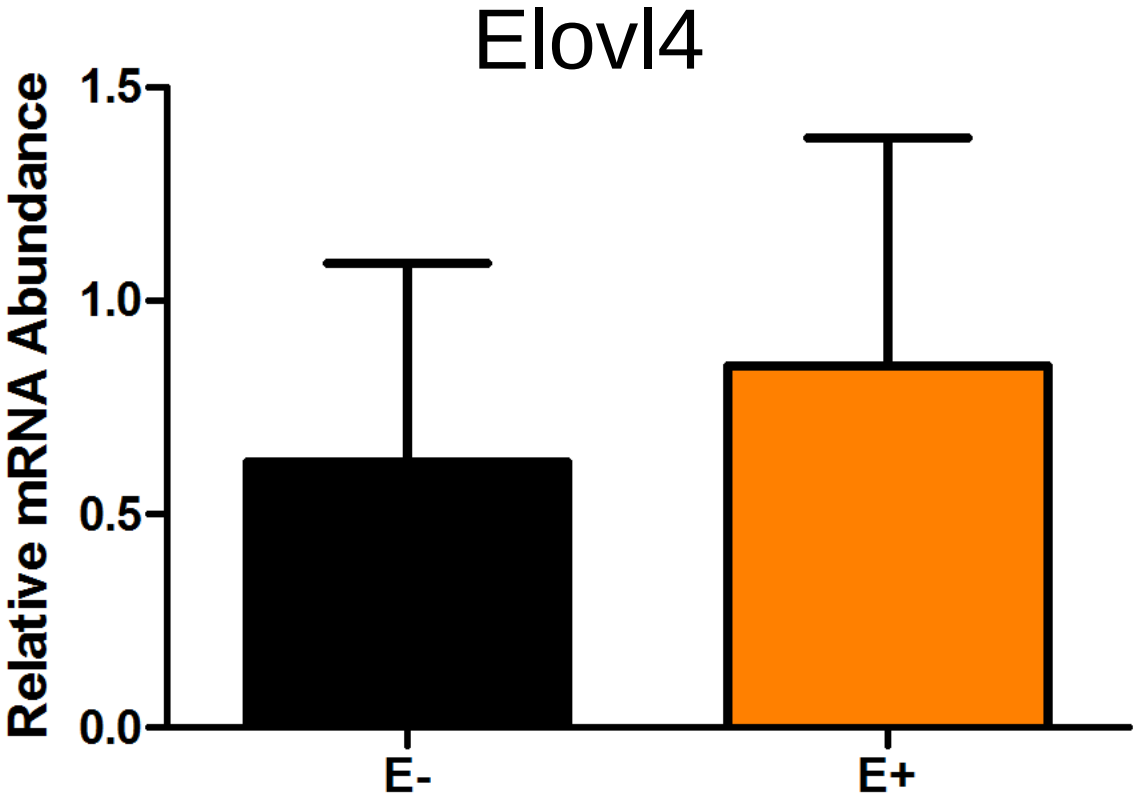


Correlation between eye DHA and α-tocopherol



R value	P value
0.5081	0.02

Vitamin E deficiency did not affect eye Elovl4 mRNA expression



Summary

- With regard to neural tissues, vitamin E deficiency in eyes (brain unstudied):
  - tended to decrease DHA levels
  - vitamin E and DHA concentrations were positively correlated
  - Other PUFA concentrations increased
- With regard to the liver, vitamin E deficiency:
  - did not change liver fatty acid concentrations

Summary, continued

- Vitamin E deficiency did not alter mRNA expression of FADs2, Elovl2, and Elovl4 in any tissues studied (brain, eye, liver)

Conclusion

- Vitamin E in the eye was not depleted sufficiently to allow DHA oxidation
  - With lower vitamin E concentrations less DHA was observed
- Vitamin E deficiency did not alter mRNA expression of FADs2, Elovl2, and Elovl4 in any tissues studied (brain, eye, liver)
- Vitamin E deficiency did not change liver fatty acid concentrations
- More samples are being analyzed

Limitations

- SREBP expression: Data not available due to western blotting optimization problems
- Data not available for DHA in the brain due to equipment failure

Acknowledgements

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