Oxylipins as an alternative way to assess dietary α-linolenic acid requirements

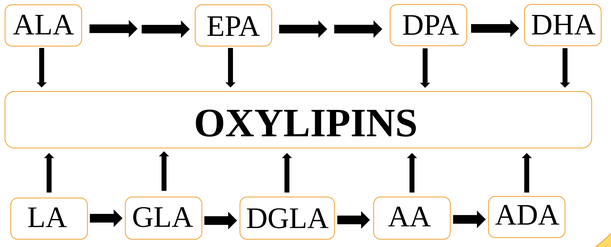
Oleksandra Fedorova, Karanbir Sidhu, Tanja Winter, Harold Aukema

University of Manitoba, Department of Food and Human Nutritional Sciences, Canadian Centre of Agri-Food Research in Health and Medicine

Introduction

•Oxylipins are hormone-like bioactive lipids derived from fatty acids (FA) (see table at right for abbreviations). They are the main effectors of FA in tissues and organisms. α-linolenic acid (ALA) is an essential FA that is required in the diet. The classical way to evaluate ALA requirements is by determining the amount of dietary ALA that maximizes DHA.

•Since oxylipins mediate the effects of FA, the effect of dietary ALA on both ω6 and ω3 oxylipins may be a more biologically relevant basis for assessing the ALA requirement. Oxylipins derived from ω6 FA generally produce pro-inflammatory oxylipins, while those derived from ω3 FA are generally anti-inflammatory lipid mediators, so decreasing the amount of ω6 while increasing ω3 oxylipins is ideal for optimal health.



Objective

Assess the dietary ALA requirement by examining what dietary level minimizes the ratio of ω6/ω3 oxylipins.

Methods

• **Animal study.** Fifty weanling male Sprague-Dawley rats were provided diets with graded levels of ALA. The higher ALA levels replaced SFA, while LA (the other essential FA) was kept constant. After 5 weeks, kidneys were harvested and stored in -80°C. Kidneys were chosen for analysis because kidney oxylipins are sensitive to dietary intervention.

• **Tissue analysis.** Lyophilized rat kidneys were homogenized with a probe homogenize. Oxylipins and free FA were extracted with SPE columns, and detected and quantified by HPLC/MS/MS.

Table 1. SFA, LA, and ALA present in each diet (n=5).

Class of FA	Amount per diet (g/100 g diet)									
LA	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
ALA	0.10	0.15	0.20	0.30	0.60	0.90	1.20	1.50	2.00	2.93
SFA	3.50	3.45	3.40	3.30	3.00	2.69	2.40	2.10	1.59	0.63

Results

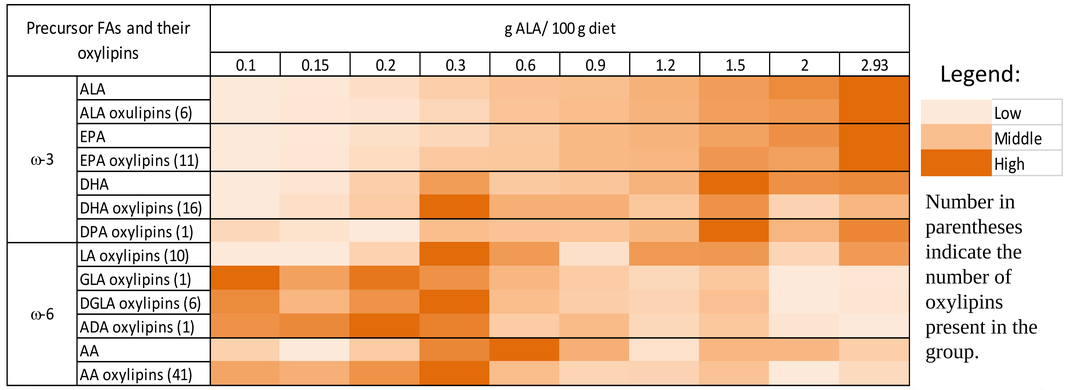


Figure 1. Heatmap showing that the concentrations (ng/mL kidney homogenate) of ω3 FA and the ω3 oxylipins increase with increasing dietary ALA. In contrast, AA and oxylipins from LA do not change, but the remaining oxylipins derived from n-6 FA decrease with increasing dietary ALA.

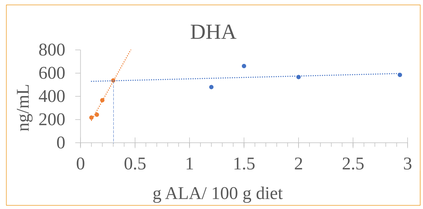


Figure 2. Classical method using DHA to assess ALA requirement shows that the maximal DHA occurs at 0.3 g ALA/100 g diet, consistent with previous estimations of the ALA requirement.

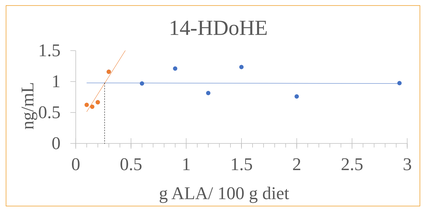


Figure 3. Example of a DHA oxylipin (14-HDoHE) showing its maximal level occurs at 0.26 g ALA/100 g diet. This is consistent with the classical estimation that uses maximal DHA as indicator.

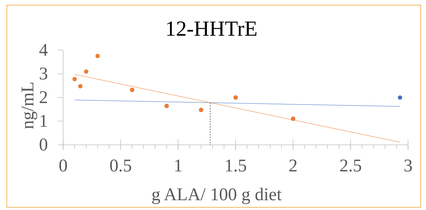


Figure 4. Example of an AA oxylipin (12-HHTrE) showing its minimal level occurs at 1.3 g ALA/100 g diet. However, this estimation was variable, making the estimate inaccurate (see Table 2).

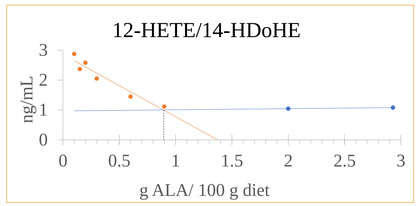


Figure 5. Example of ratio of AA/DHA oxylipins (12-HETE/14-HDoHE) showing its minimal level occurs at 0.80 g ALA/ 100 g diet. Despite being based on just 6 ratios this estimation is more consistent (see Table 2)

Indicator (n of oxylipins)	g ALA mean	g ALA range	Coefficient of Variation
DHA (classical) (1)	0.3	-	-
DHA oxylipins (16)	0.26	0.16-0.35	0.13
AA oxylipins (41)	1.31	0.81-2.28	0.55
AA/DHA oxylipins (6 ratios)	0.79	0.58-0.91	0.31

Table 2. Estimation of ALA requirements based on DHA, AA and AA/DHA oxylipins showing an average of 0.58-0.91 g ALA/100 g diet based on AA/DHA oxylipins.

Results and Conclusions

Figure 1 shows that increasing dietary ALA increases ω3 FA and oxylipins and decreases most ω6 oxylipins.

Figures 2 and 3: Using the classical approach and DHA oxylipins, estimated ALA requirement is consistent with the current dietary requirement of 0.25-0.3 g ALA/100 g.

Figure 4 and Table 2: The effect of dietary ALA on reducing AA derived oxylipins is subtle and variable, making estimation of requirement inaccurate.

Figure 5: The ratio of ω6/ω3 oxylipins markedly reduced this variability and provided a more consistent estimation of the ALA requirement.

Conclusion: Based on the ω6/ω3 oxylipin ratio, the dietary ALA requirement is estimated to be 0.58-0.91 g/100 diet for the growing rat. This is higher than the current requirement of 0.25-0.3g ALA/100g diet. Further studies are needed to refine this estimate.

Abbreviation

AA = Arachidonic Acid; 20:4ω-6
ADA = Adrenic acid; 22:4ω-6
ALA = α-linolenic acid; 18:3ω-3
DGLA = Dihomo-γ-linolenic acid; 20:3ω-6
DHA = Docosahexaenoic acid; 22:6ω-3
DPA = Docosapentaenoic acid; 22:5ω-3
EPA = Eicosapentaenoic acid; 20:5ω-3
FA – fatty acid
GLA = Gamma-linolenic acid; 18:3ω-6
HPLC/MS/MS= High Performance Liquid Chromatography/ Tandem Mass Spectrometry
LA = Linoleic acid; 18:2ω-6
SFA = Saturated Fatty Acids
SPE = Solid Phase Extraction

Significance and Future Directions

Significance:

- This approach to estimating the dietary ALA requirement results is **higher** than previously assessed by the traditional method.

Future Directions:

- Assess the dietary ALA requirement based on blood and other tissues, and in females, adults, during pregnancy and ultimately in humans.
- Determine the equivalent level of dietary EPA and/or DHA that achieves the same minimal levels of AA/DHA oxylipins compared to dietary ALA alone.
- Determine the effect of dietary LA on the ALA requirement.

Funding

• Supported by the U of M USRA program and NSERC