Effect Of Dietary Polyunsaturated Fatty Acids On Rat Tissue Oxylipin Profiles

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Abstract

There is much information on the effects of dietary lipids on tissue fatty acid composition, but little data on their bioactive lipid metabolites that mediate their effects on health and disease. Therefore diets differing in levels of linoleic (LA), α-linolenic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were provided to male and female weanling rats for 6 weeks, and fatty acids and their oxygenated metabolites (oxylipins) were quantified in kidney, liver and serum. The findings reveal that although oxylipin profiles are generally similar to fatty acid profiles there are some notable exceptions: dietary LA can increase LA and arachidonic acid derived oxylipins without changing the precursor fatty acid level; dietary ALA can increase DHA oxylipins without changing DHA levels. Male tissues generally had higher levels of oxylipins than females, with a few exceptions (e.g. F-series prostaglandins). These findings have implications for dietary recommendations for LA and n-3 fatty acids.

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Abbreviations

Δ	Delta
%E	Percentage of energy
AA	Arachidonic acid
AdA	Adrenic acid
ADA	American Diabetes Association
ALA	α-linolenic acid
BHT	Butylated hydroxy toluene
COX	Cyclooxygenase
cPLA$_2$	Cytosolic phospholipase A_2
CYP	Cytochrome P450
20 COOH AA	20 carboxy arachidonic acid
DγLA	Dihomo-gamma-linolenic acid
dh	Dehydro
dhk	Dihydro-keto
DiHDoHE	Dihydroxy-docosahexaenoic acid
DiHDoPE	Dihydroxy-docosapentaenoic acid
DiHETE	Dihydroxy-eicosatetraenoic acid
DiHETrE	Dihydroxy-eicosatrienoic acid

DiHODE	Dihydroxy-octadecadienoic acid
DiHOME	Dihydroxy-octadecenoic acid
DPA	Docosapentaenoic acid
EDA	Eicosapentaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
EpDPE	Epoxy-docosapentaenoic acid
EpETE	Epoxy-eicosatetraenoic acid
EpETrE	Epoxy-eicosatrienoic acid
EpODE	Epoxy-octadecadienoic acid
EpOME	Epoxy-octadecenoic acid
ELOVL	Elongation of very long-chain fatty acid
Ex	Eoxin
FADS	Fatty acid desaturase
GC	Gas chromatography
GLA	gamma-linolenic acid
HDoHE	Hydroxy-docosahexaenoic acid
HEPE	Hydroxy-eicosapentaenoic acid
HETE	Hydroxy-eicosatetraenoic acid

HETrE	Hydroxy-eicosatrienoic acid
HHTrE	Hydroxy-heptadecatrienoic acid
HEPE	Hydroxy-eicosapentanoic acid
HDoHE	Hydroxy-docahexanoic acid
HODE	Hydroxy-octadecadienoic acid
HOTrE	Hydroxy-octadecatrienoic acid
HpDoHE	Hydroperoxy-docosahexaenoic acid
HpETE	Hydroperoxy-eicosatetraenoic acid
HPLC	High performance liquid chromatography
Hx	Hepoxilin
k	Keto
LA	Linoleic acid
LC/MS	Liquid chromatography/mass spectrometry
LOX	Lipoxygenase
Lt	Leukotriene
LX	Lipoxin
MUFA	Monounsaturated fatty acid
NE	Nonenzymatic products
OH	Hydroxy

oxo-ETE	Oxo-eicosatetraenoic acid
oxo-ODE	Oxo-octadecadienoic acid
oxo-OTrE	Oxo-octadecatrienoic acid
PG	Prostaglandin
PD	Protectin
PGEM	Prostaglandin E metabolite
PGDM	Prostaglandin D metabolite
PUFA	Polyunsaturated fatty acid
PDX	10(S), 17(S)- dihydroxy-docosahexaenoic acid
sEH	Soluble epoxide hydrolase
SFA	Saturated fatty acid
SPE	Solid phase extraction
TLC	Thin-layer chromatography
TriHOME	Trihydroxy-octadecenoic acid
TX	Thromboxane
TrX	Trioxilin
UFA	Unsaturated fatty acid
Rv	Resolvin

Contributions To Thesis:

Shan Leng- diet preparation, animal care, provision of diet to animals, body weight recording, termination, tissue weighing and collection; lyophilized, pulverized and homogenized all tissues for oxylipin and fatty acid analysis; extracted, separated, and methylated fatty acids from all tissues and serum, analyzed the chromatograms and quantified the fatty acid composition; extracted and isolated all oxylipins, prepared oxylipin samples on the HPLC-MS/MS, analyze all the chromatographs on Multi-Quant, quantified the oxylipins; performed all calculations for product/pre-cursor ratios, performed the statistical analyses for all results; wrote the chapter and created all tables and figures.

Tanja Winter- ran the internal standards on the HPLC-MS/MS to determine the m/z ratios to identify the analytes in the samples; helped with education with the HPLC-MS/MS, calculated the deuterated standards amount that should be added in the samples; did the slopes used for quantifying oxylipins.

Dennis Labossiere and Dennis Joseph-ran fatty acid samples on GC/MS.

Melissa Gabbs, Jessay Devassy, Monir Monirujjaman, Harold Aukema -the co-authors of the published review paper which is used in parts of my literature review. Melissa Gabbs and Shan Leng were equally contributing first authors on this paper.

Chapter 1 Literature Review

1.1 Introduction

Oxylipins are polyunsaturated fatty acid (PUFA) oxidation products formed via one or more mono- or di-oxygen dependent reactions. They are major mediators of PUFA effects in the body, with the most well-known oxylipins being the eicosanoids formed from arachidonic acid (AA). Oxylipins also can be formed from other PUFA, with the more common ones being octadecanoids derived from linoleic acid (LA) and α-linolenic acid (ALA), eicosanoids derived from dihomo-γ-linolenic acid (DGLA) and eicosapentaenoic (EPA), and docosanoids derived from adrenic acid (AdA) and docosahexaenoic acid (DHA). The PUFA precursors to oxylipins can be obtained directly from the diet or from the elongation and desaturation of LA and ALA into longer chain PUFA. Hence, a high n-6 PUFA intake is generally associated with a high level of n-6 PUFA derived oxylipins and a high n-3 PUFA intake is generally associated with high level of n-3 PUFA derived oxylipins.

However, the types of oxylipins produced from tissue PUFA not only depend on the level of dietary PUFA consumed, but also on the levels of competing PUFA for incorporation into phospholipid and for elongation and desaturation to longer chain PUFA. Further, the oxygenases present for metabolizing these PUFA into oxylipins in each tissue, as well as enzyme preferences for specific PUFA influences oxylipin production. Hence the tissue oxylipin profile does not necessarily mimic the dietary PUFA intake or the tissue PUFA profile, necessitating the direct assessment of the tissue oxylipins in order to understand the effects of PUFA that are mediated via oxylipins. The recent advent of lipidomics methodologies has enabled the analyses of oxylipin profiles from all PUFA substrates simultaneously, raising the awareness of the vast number of oxylipins in the body. Indeed, these analyses have shown that AA oxylipins comprise less than half of all oxylipins. Other studies have shown that oxylipins derived from PUFA

besides AA also have significant biological activity. This necessitates the investigation of the entire oxylipin profile in order to understand the overall effects of dietary PUFA via their metabolism to oxylipins.

There are debates about LA, ALA, EPA and DHA intake. LA intake has increased over the last half-century and there are many different recommendations for LA intake. The debates surrounding LA mainly are focused on if LA can be converted to AA oxylipins. DHA and EPA are known to have beneficial effects for health and that their derived oxylipins play critical roles in maintaining health. However there is no specific recommendation for EPA or DHA intake. Debates of n-3 fatty acid intake also are focused on whether ALA is sufficient to provide benefits from longer chain n-3 fatty acids. Evidence shows that with a higher ALA intake, DHA level is not increased in most cases. But it is not known whether DHA and EPA oxylipins will increase or not. Therefore, since there is currently no collated data on oxylipins in mammalian tissue, the purpose of this project is to examine the effect of different PUFA on oxylipin production.

1.2 Oxylipin Formation

Oxylipins are found throughout the body in all tissues, urine and blood. Classically they have been described as having a short half-life, acting locally and not being stored, but being synthesized *in situ* when needed. However, not all oxylipins are short-lived, as evidenced by the steady-state levels of both free and esterified oxylipins in tissues such as the liver, adipose, kidney, ileum, etc. (1-3). The free forms are presumably the biologically active oxylipins, but the functions of those that are found esterified to phospholipid are not known. It is possible that they may alter membrane properties or act as a storage reservoir.

Oxylipin formation begins with cell activation, which results in precursor PUFA in the sn-2 position of membrane phospholipids being liberated by cytosolic phospholipase

A_2 ($cPLA_2$) (4). Evidence for the importance of this enzyme is provided by findings from a patient lacking this enzyme, in whom liberation of free PUFA and subsequent oxylipin formation is reduced compared to healthy controls (5, 6). However, even though only AA oxylipins were examined in these studies, lack of $cPLA_2$ did not completely block oxylipin formation. A recent study showed that inhibition of adipose triacylglyceride lipase in mast cells also reduced oxylipin formation (7). Since triacylglycerides typically contain only small amounts of AA, it raises the question of whether non-AA PUFA might be released in greater amounts via alternate pathways, such as adipose triglyceride lipase. Further studies examining whether PUFA liberation via this enzyme is a direct source of PUFA for oxylipin biosynthesis or whether it indirectly provides PUFA for incorporation into phospholipid prior to liberation via $cPLA_2$ activity, remain to be carried out. Once formed, free oxylipins can mediate their biological effects via interactions with receptors or intracellular effectors, or can be re-esterified into lipids. In addition, small amounts of PUFA esterified to phospholipid or cholesterol can be converted into oxylipins *in situ* (8, 9).

PUFA metabolism into oxylipins occurs by three main pathways, which are briefly described below. For more details on specific oxylipin generating enzymes, oxylipin receptors and breakdown products of oxylipins there are several excellent reviews (10-21).

Cyclooxygenase. The first oxylipin generation pathway involves cyclooxygenase (COX) enzymes, which convert PUFA into prostanoids – i.e. prostaglandins and thromboxanes (10-12). Prostanoids have one or more double bonds and a characteristic five-carbon ring structure at the 8- to 12-carbon positions of 20-carbon PUFA derived oxylipins. COX converts DGLA, AA, EPA and AdA into 1-, 2-, 3- and dihomo-2-series prostanoids, such as prostaglandin D_1 (PGD_1), PGD_2, PGD_3 and dihomo-PGD_2, respectively (22, 23). After the prostanoids are produced and released, they mediate their effects via binding to G protein-coupled receptors on the surface of cells, or other

intracellular effectors, such as peroxisome proliferator-activated receptor γ (PPARγ) (10, 12). The number of double bonds and the type of ring structure of a prostanoid determines its receptor specificity. There are five classes of prostanoid receptors, including receptors for PGD, PGE, PGI, PGF and thromboxane A (TxA). Each of these receptors can have several isoforms, which may themselves have differing effects. They are characterized by their most potent biological ligand, but there is also some ligand cross-reactivity with these receptors (12). In addition to the prostanoids, COX also can produce select hydroxy fatty acids [e.g. 11-hydroxy-eicosatetraenoic acid (11-HETE) from AA; 13-hydroxy-docosahexaenoic acid (13-HDoHE) from DHA; 9-hydroxy-octadecadienoic acid (9-HODE) from LA] (24-27).

Lipoxygenase. The second pathway of oxylipin formation involves lipoxygenases (LOX) that catalyze the formation of hydroxy fatty acids and their metabolites (including leukotrienes, lipoxins, resolvins, protectins, maresins, hepoxilins and eoxins). There are multiple LOX enzymes that have traditionally been classified by the position of the hydroperoxy and hydroxy fatty acid they form from AA (e.g. 5-HpETE and 5-HETE are formed from AA by 5-LOX activity). This nomenclature has limitations because the position is different with PUFA of differing chain length, some enzymes act at multiple positions, and there can be differences in the positional specificities of the same homolog in different species (11, 15). An alternative nomenclature is to use the gene names to describe the LOX enzymes (15).

Hydroxy fatty acids (e.g. 5-HETE) produced via LOX are further metabolized to their keto [(e.g. oxo-eicosatetraenoic acid (oxo-ETE)] or dihydroxy (e.g. 5,15-DiHETE) derivatives. 5-LOX activated by 5-LOX activating protein (FLAP) results in the production of leukotrienes, including leukotriene B_4 (LtB_4) and those previously known as the slow reacting substance of anaphylaxis, the cysteinyl leukotrienes (19). Combinations of sequential LOX activities (and sometimes including epoxygenase and hydrolase activities) results in the formation of di- and tri-hydroxy fatty acids, which

includes the lipoxins, resolvins, protectins and maresins (14, 16). Hepoxilins also are formed from 12-HpETE (21) and eoxins from 15-HpETE (28). As with prostanoids, the LOX-derived oxylipins also appear to mediate their effects via binding to G protein-coupled receptors and intracellular effectors, although receptors for all oxylipins have not been identified.

Cytochrome P450. The third pathway of PUFA metabolism to oxylipins involves a diverse array of membrane bound cytochrome P450 (CYP) enzymes that are so named because of their unique absorbance at 450 nm when reduced and bound by carbon monoxide. Originally known for their roles in xenobiotic metabolism, there are over 50 CYP enzymes expressed in humans, divided into multiple families and subfamilies based on amino acid identity (11). CYP enzymes that form oxylipins can have epoxygenase or ω-hydroxylase activity. For example, they can convert AA, EPA and DHA into epoxy-eicosatrienoic acid (EpETrE, also abbreviated as EET), epoxy-eicosatetraenoic acid (EpETE, also abbreviated as EEQ) and epoxy-docosapentaenoic acid (EpDPE, also abbreviated as EDP), respectively, via epoxygenase, and HETE, hydroxy-eicosapentaenoic acid (HEPE) and HDoHE, respectively, via ω-hydroxylase activity. Epoxygenase products are rapidly metabolized via soluble epoxide hydrolase (sEH) to form dihydroxy fatty acids, such as the AA, EPA and DHA metabolites, dihydroxy-eicosatrienoic acid (DiHETrE), DiHETE and dihydroxy-docosapentaenoic acid (DiHDPE), respectively. Similar to oxylipins formed via the other pathways, these oxylipins also mediate their effects via specific receptors or by cross-reacting with other oxylipin receptors (11, 13, 17, 18). In addition, they may also enter cells and mediate effects intracellularly by modulating transcription factors and ion channels (13).

1.3 PUFA Substrates For Oxylipin Formation

Oxylipins are formed from a number of n-3 and n-6 PUFA precursors, such as the n-6 PUFA AA, LA, gamma-linolenic acid (GLA), DGLA and AdA, and the n-3 PUFA ALA, stearidonic acid (SDA), EPA and DHA. Although studies indicate that $cPLA_2$ exhibits preference for AA and EPA (29, 30), the presence of oxylipins from other PUFA demonstrates that they can be released in sufficient quantities for oxylipin production.

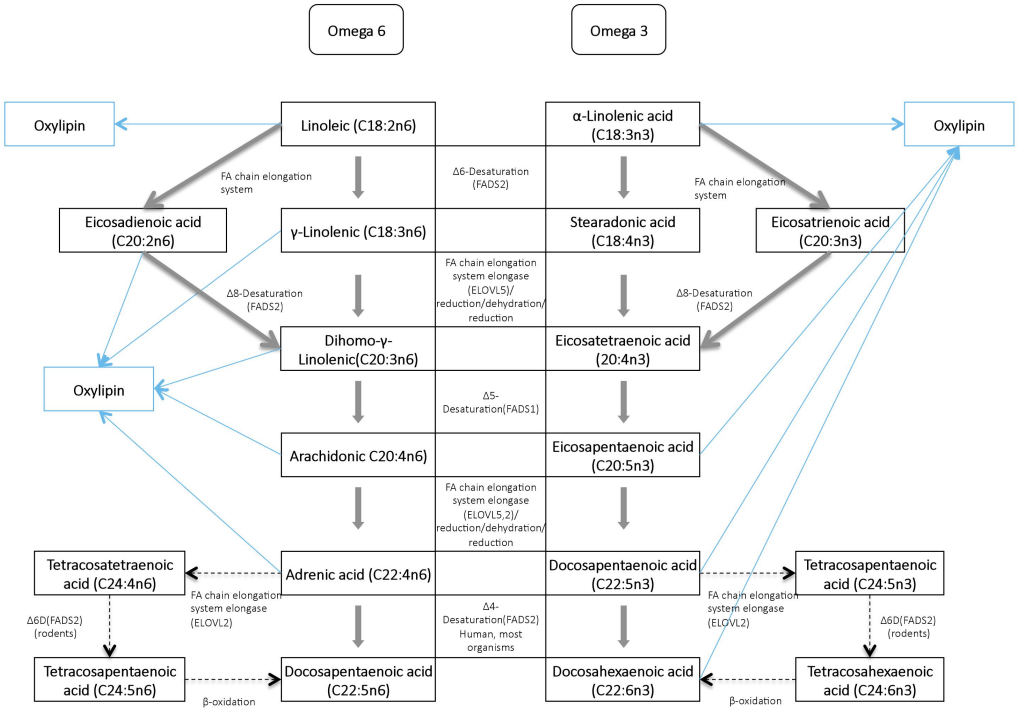
The fatty acid metabolism pathway is shown in Figure 1.1. As shown, LA is desaturated to form DGLA, followed by elongation to GLA, and then further desaturation to AA. Further elongation and desaturation results in the formation of AdA, which is usually found in lower amounts in tissues.

The same enzymes catalyze the conversion of ALA into EPA, DPA (n-3), and DHA. They are the forms of n-3 fatty acids in the body. As a result of using these enzymes in common, competition between n-3 and n-6 fatty acid metabolism can occur. Excess LA inhibits ALA conversion into EPA and DHA (31). GLA conversion to DGLA is very rapid (32), so the main forms of n-6 fatty acid in body are primarily LA, DGLA and ARA. When essential n-3 and n-6 FA intake is limited, n-7 and n-9 PUFA will accumulate in their place.

N-6 PUFA

Arachidonic Acid AA produces 2-series oxylipins (Figure 1. 2) via the COX pathway, initially resulting in formation of PGG_2 and PGH_2, which is then rapidly converted to other prostaglandins (e.g. $PGF_{2\alpha}$) and thromboxanes (e.g. TxA_2) via specific prostaglandin and thromboxane synthases (20). As is the case with the other oxylipins, prostanoids are then rapidly degraded to numerous inactive and active metabolites, some of which can be used as markers of the parent compound, while others can mediate the same or opposite effects ascribed to the parent compounds (33-35).

Figure 1.1. Fatty Acid Biosynthesis Pathway.



Fatty acid desaturase (FADS) and elongation of very long-chain fatty acid (ELOVL) catalyzing both n-3 and n-6 fatty acid for desaturation and elongation, and that n-3 fatty acid and n-6 fatty acid compete for those enzymes. This figure is adapted from Zhang et,al (36). The solid thick line indicates the main metabolism pathway, the solid thin line indicates the substrate fatty acid to produce oxylipins, the dotted line indicates an alternative metabolism pathway.

Figure 1.2. Arachidonic Acid–Derived Oxylipins.

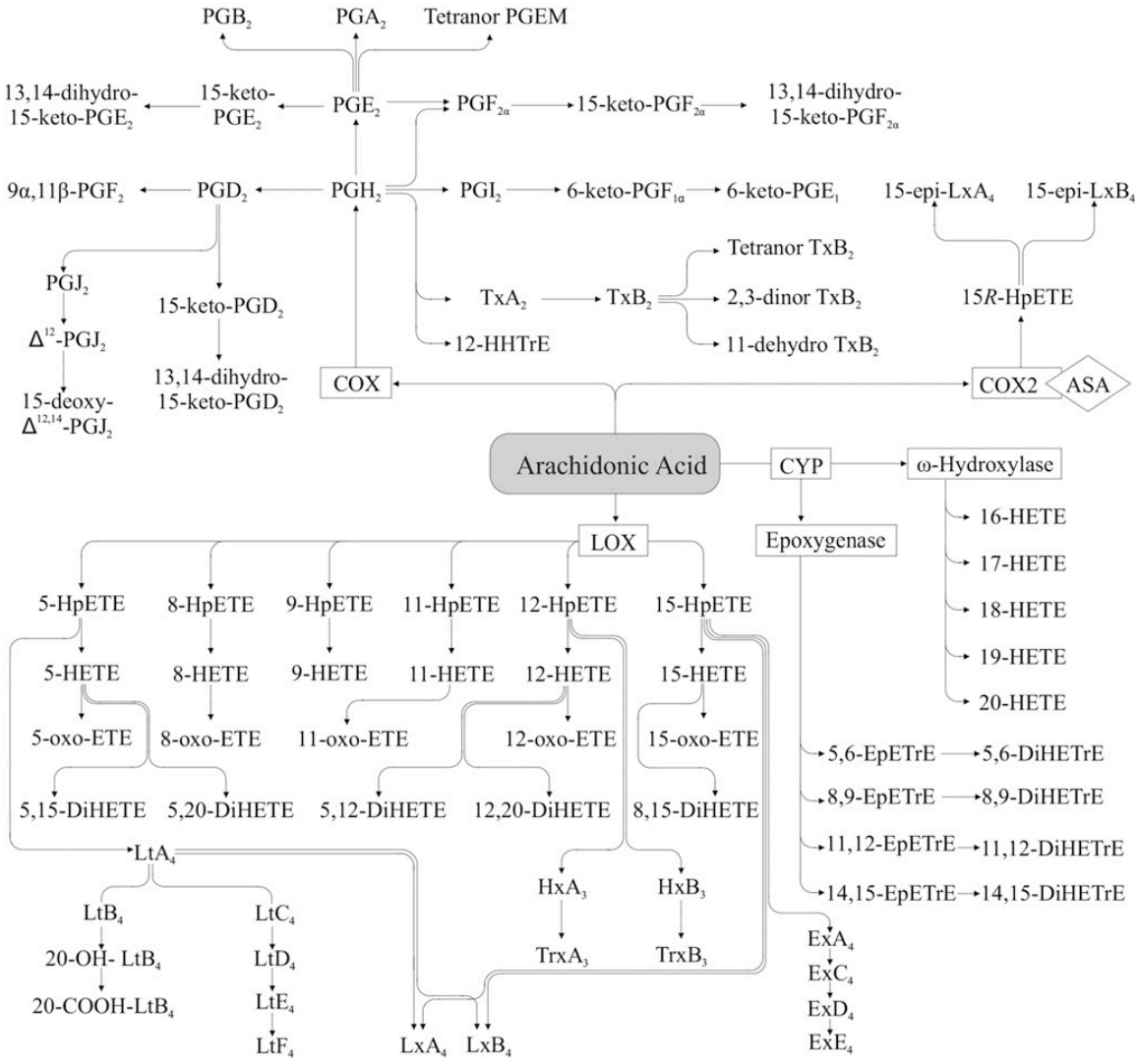


Figure from published review (44) from our laboratory, of which I was co-first authour.

AA also produces oxylipins via the LOX pathway, resulting in HpETE, (e.g. 12-HpETE), which are further rapidly converted to hydroxy fatty acids via glutathione peroxidase (37). 5-, 12-, 15-HETE are the most commonly described HETE in mammals, although 8-, 9- and 11-HETE also are produced, and sometimes in greater amounts (38, 39). The 11- or 15-HETE isomers also can be produced via COX activity, as indicated above (24, 25). The HETE can be further converted to oxo-ETE via dehydrogenase activity (40, 41), or to DiHETE, via further COX (e.g. 5,11-DiHETE), LOX (e.g. 5,15-DiHETE) or CYP ω-hydroxylase (e.g. 5,20-DiHETE) activity (42, 43). In addition, the HpETE formed via LOX can be metabolized via several other routes: 5-HpETE can be further converted to 4-series leukotrienes (e.g. LtC_4), via 5-LOX after activation by FLAP; 12-HpETE can be isomerized to hepoxilins (e.g. HxB_3) and subsequently converted to trioxilins [e.g. trioxilin B_3 ($TrxB_3$)] (21, 44); and 15-HpETE can be converted to eoxins (e.g. ExC_4) (28). As well, lipoxins (e.g. LxA_4) can be formed from 5- or 15-HpETE via further LOX activity (45-47). Epi-Lx (e.g. 15-epi-LxA_4) formation can also be initiated by aspirin acetylated or nitrosylated COX2 and 5-LOX (48-50). AA also can be converted non-enzymatically to HETE (51) and isoprostanes (e.g. iso-$PGF_{2\alpha}$) (52). The latter are often used as a marker of oxidative stress in vivo; for further discussion of these non-enzymatic oxylipins, see review in (52).

AA metabolism via CYP ω-hydroxylase activity results in the formation of HETE with the hydroxy group being at the omega or methyl end of the fatty acid (e.g. 20-HETE), while CYP epoxygenase activity yields epoxy fatty acids (e.g. 14,15-EpETrE), which can be converted to dihydroxy fatty acids (e.g. 14,15-DiHETE), via sEH activity, as reviewed in (13, 17, 18). Formation of other HETE (e.g. 13-HETE) may be mediated via CYP bisallylic hydroxylase activity (53-55), but the importance of this pathway is less known.

Linoleic Acid Even though the size of the literature for LA oxylipins is markedly smaller than for most other oxylipins (especially AA oxylipins) (Figure 1. 3.), they are usually present in tissues and blood in higher amounts than oxylipins derived from any other PUFA (56-58). LA produces oxylipins through the LOX pathway, resulting in hydroperoxy fatty acids, which are rapidly converted to hydroxy fatty acids [e.g. 13-hydroxy-octadecadienoic acid (HODE)], which can be further metabolized to keto fatty acids [e.g. 13-oxo-octadecadienoic acid (13-oxo-ODE) (59, 60). LA also can be metabolized via the epoxygenase activity of CYP, resulting in epoxygenated fatty acids [e.g. 9,10-epoxy-octadecenic acid (9,10-EpOME)], which are metabolized via sEH activity to form dihydroxy fatty acids [e.g. 9,10-dihydroxy-octadecenoic acid (9,10-DiHOME)] (61). Further, LA can be converted to trihydroxy fatty acids [e.g. 9,10,13-trihydroxy-octadecenoic acid (9,10,13-TriHOME)] potentially by sequential metabolism of LOX and epoxygenase activity and/or auto-oxidation (62). Several other LA oxylipins also can be produced non-enzymatically (e.g. 9-HODE) (63). There also are reports that the formation of a small amount of the LA oxylipins may be mediated via COX (e.g. 9-HODE) (27, 64) or CYP bisallylic hydroxylation (e.g. 17-HODE) (53-55) activity; the relative importance of these pathways remain to be elucidated.

γ-Linolenic Acid. GLA can be converted via LOX to 10- and 13-hydroxy-octadecatrienoic acid(γ) [13-HOTrE(γ)] (65) in human platelets and via CYP to γ-6,7-, γ-9,10- and γ-12,13-epoxy-octadecadienoic acid (γ-12,13-EpODE) by human CYP enzymes in vitro (66). Other oxylipins derived from GLA (e.g. 6-HOTrEγ) have been reported to be synthesized in vitro in a patent application (67). Note that oxylipins derived from GLA are distinguished from ALA oxylipins with the use of the γ notation.

Figure 1.3. Linoleic Acid–Derived Oxylipins.

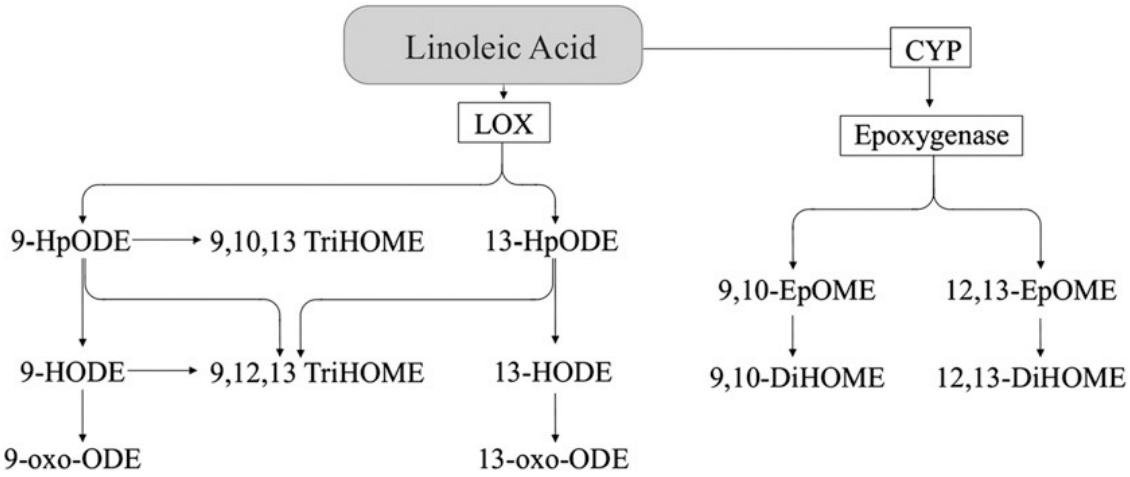


Figure from published review (44) from our laboratory, of which I was co-first authour.

Dihomo-γ-Linolenic Acid DGLA can be converted via COX to 1-series prostaglandins (Figure 1. 4) (e.g. PGI_1) and thromboxanes (e.g. TxA_1) (22, 68, 69), via LOX to yield hydroperoxy (e.g. 15-HpETrE) and hydroxy fatty acids [e.g. 15-hydroxy-eicosatrienoic acid (15-HETrE)] (70-75), and via CYP epoxygenase and sEH to epoxy-eicosadienoic acid (EpEDE) (e.g. 8,9-EpEDE) and dihydroxy-eicosadienoic acid (DiHEDE) (e.g. 8,9-DiHEDE) (71, 72, 76).

Adrenic Acid AdA can be metabolized by COX into dihomo-prostaglandins such as dihomo-PGE_2, dihomo-TxB_2, and dihomo-PGI_2 (Figure 1. 5) (77-82). Metabolism via the LOX pathway generates hydroxy-docosatetraenoic acids (also referred to as dihomo-HETE) such as 17-hydroxy-docosatetraenoic acid (dihomo-17-HETE), which can be further converted to dihydroxy compounds (e.g. dihomo-10,17-DiHETE) (79-81), and via the CYP pathway to dihomo-EpETrE (epoxy-docosatrienoic acids) such as dihomo-16,17-EpETrE, which can be further converted to their respective dihydroxy compounds e.g. (dihomo-16,17-DiHETrE) (79).

Figure 1.4. Dihomo- γ -Linolenic Acid–Derived Oxylipins.

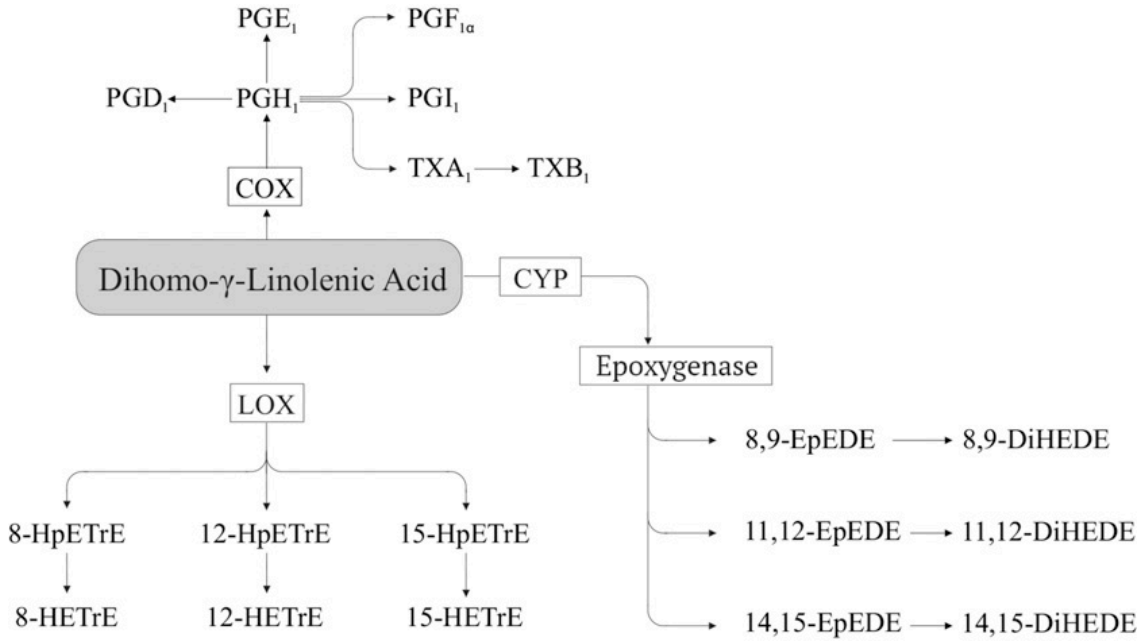


Figure from published review (44) from our laboratory, of which I was co-first authour.

Figure 1.5. Adrenic Acid– Derived Oxylipins.

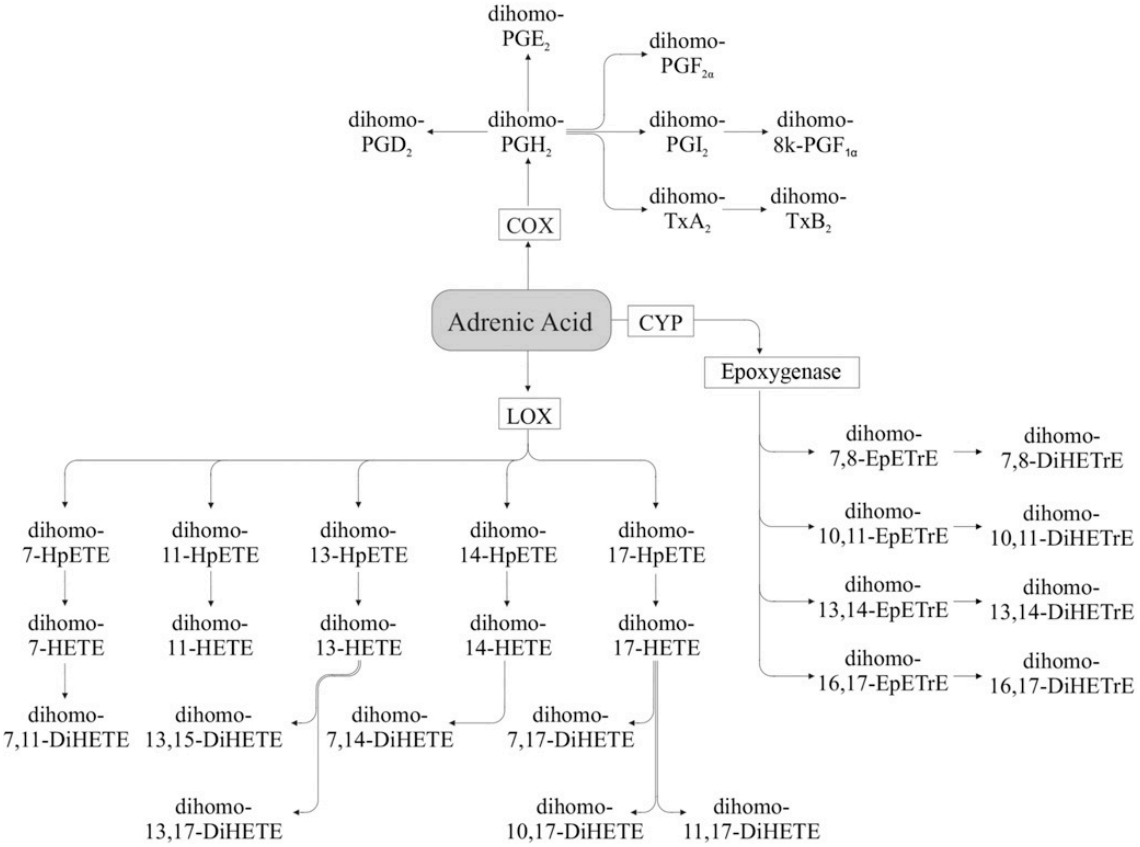


Figure from published review (44) from our laboratory, of which I was co-first authour.

N-3 PUFA

α-Linolenic Acid ALA produces oxylipins via the LOX pathway, resulting in hydroxy fatty acids, (e.g. 9-HOTrE), which can be further metabolized to keto fatty acids [e.g. 9-oxo-octadecatrienoic acid (9-oxo-OTrE)] (Figure 1. 6) (83). As with LA, there are reports that indicate that HOTrE may be formed via COX activity, but the importance of this pathway in vivo remains to be determined (27). ALA also can be metabolized via CYP epoxygenase activity, resulting in epoxygenated fatty acids, (e.g. 12,13-EpODE) (66), which can be further converted to dihydroxy fatty acids [e.g. 12,13-dihydroxy-octadecadienoic acid (12,13-DiHODE)] via sEH activity (57). Other ALA metabolites that have been reported include 18-HOTrE from ALA via CYP activity (18), 9,16-DiHOTrE via LOX activity (83) and 12-HOTrE via COX2 activity (27).

Stearidonic Acid. Oxylipins derived from SDA (e.g. 13- hydroxy-octadecatetraenoic acid) have been reported to be produced in vitro in a patent application (67).

Eicosapentaenoic Acid Similarly to AA, EPA produces oxylipins via the COX pathway, yielding 3-series prostaglandins (e.g. PGE_3) and thromboxanes (e.g. TxA_3) (23) (Figure 1. 7). EPA compared to AA is generally a poorer substrate for COX, particularly for the COX1 isoform (84). EPA can produce hydroperoxy fatty acids (e.g. 5-HpEPE), which can be further converted to hydroxy fatty acids (e.g. 5-HEPE) by LOX activity (23, 85, 86), and 5-series leukotrienes (e.g. LtB_5) via combined 5-LOX and FLAP activity (86, 87). HEPE such as 5-HEPE also can be metabolized to dihydroxy-eicosapentaenoic acids (DiHEPE) such as 5,12-DiHEPE (88) or to keto fatty acids such as 5-oxo-EPE (89). Metabolites of other HEPE isomers are likely to be present, but few have been identified. Hydroxy fatty acids from EPA with hydroxy groups on the 18-20-carbon positions also are formed via ω-hydroxylase activity of the CYP pathway (e.g. 18-HEPE) (90, 91). The 18-HEPE formed via this pathway (as well as by acetylated COX2) can be further converted to the E-series resolvins [e.g. resolvin E1 (RvE1)] via 5-LOX activity(43, 46, 92). EPA can also produce epoxy fatty acids (e.g. 14,15-EpETE) via CYP epoxygenase

Figure 1.6. α-Linolenic Acid–Derived Oxylipins.

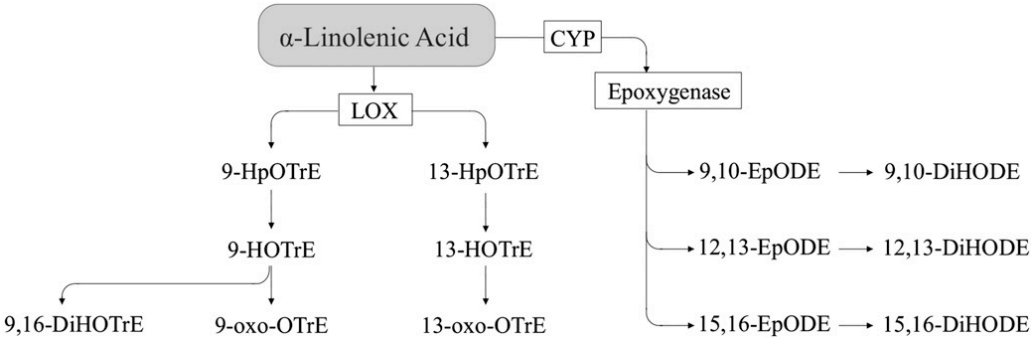


Figure from published review (44) from our laboratory, of which I was co-first authour.

Figure 1.7. Eicosapentaenoic Acid -Derived Oxylipins.

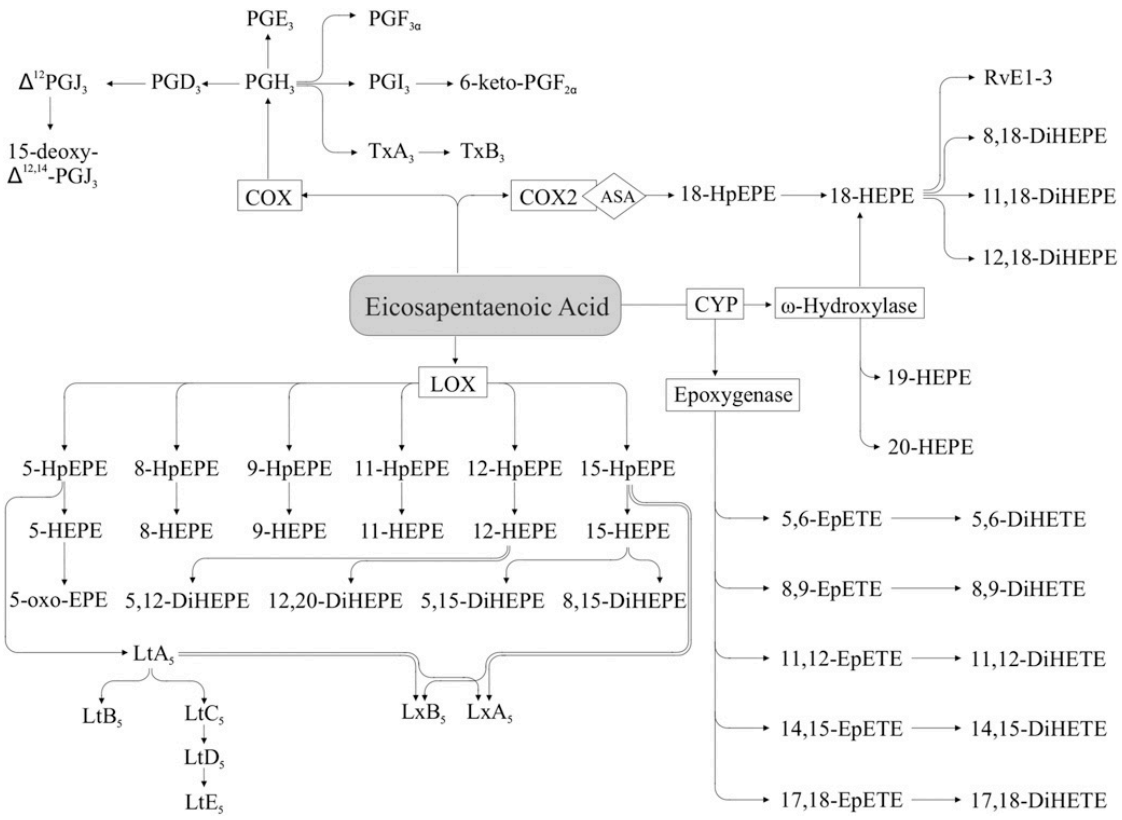


Figure from published review (44) from our laboratory, of which I was co-first authour.

activity (93), which can be further converted to dihydroxy fatty acids (e.g. 14,15-DiHETE) by sEH (94). As with AA and LA, bisallylic hydroxylation of EPA can also yield HEPE such as 10-HEPE (95).

Docosahexaenoic Acid. DHA can be metabolized via the LOX pathway to hydroxy fatty acids (e.g. 4-HDoHE), with a hydroperoxy intermediate (e.g. 4-HpDoHE) (96) (Figure 1. 8). The hydroperoxy 14-HpDoHE can be further metabolized to form maresins (e.g. MaR1) (97), and 17-HpDoHE can be metabolized to 17-HDoHE, or to resolvins (e.g. RvD1) and protectins [e.g. protectin D1 (PD1)] via further LOX and epoxygenation steps. PD1 is produced via LOX, epoxide formation from the hydroperoxide product, and epoxide hydrolase activity (98) while PDX is formed via double LOX activity (99). 17-HpDoHE derived from DHA also can be produced via aspirin acetylated COX2, yielding the aspirin-triggered (AT)-resolvins (e.g. AT-RvD1) and -protectins (e.g. AT-PD1) (26, 100, 101). DHA also has been shown to yield hydroxy fatty acids non-enzymatically (e.g. 8-HDoHE) (102, 103) and 13-HDoHE can be formed via COX2 (26). Recent studies provide evidence that HDoHE also can be metabolized to dihydroxy (e.g. 14,20-DiHDoHE) (104) and keto fatty acids (e.g. 7-oxo-DoHE) (105) with more likely to be demonstrated in the future. Oxylipins can be produced from DHA via CYP epoxygenase activity, yielding epoxy fatty acids (e.g. 16,17-EpDPE) (93, 96), which can be converted to dihydroxy fatty acids (16,17-DiHDPE) via sEH (94). CYP ω-hydroxylase activity produces HDoHE with hydroxy groups near the methyl end of DHA (e.g. 21-HDoHE) (96).

Figure 1.8. Docosahexaenoic Acid -Derived Oxylipins.

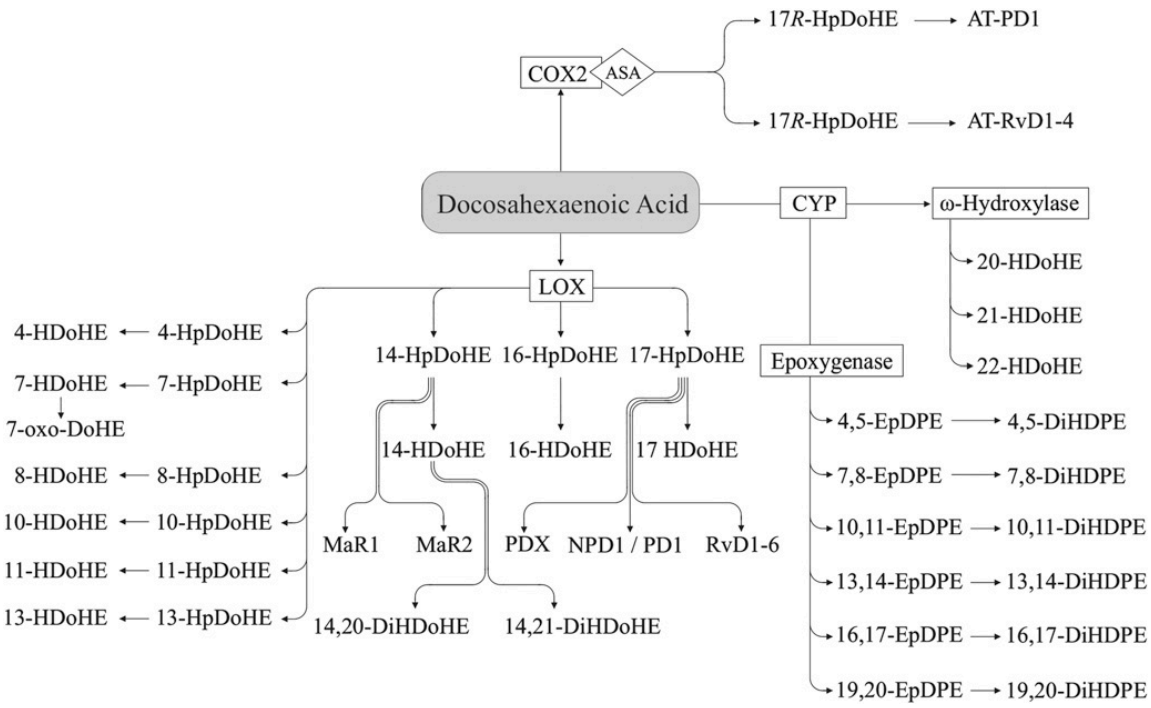


Figure from published review (44) from our laboratory, of which I was co-first authour.

1.4 Oxylipin Functions

Oxylipins have a wide range of functions, many of which are still being elucidated. In addition, oxylipins derived from different pathways, as well as different substrate PUFA can have similar or opposing effects, necessitating knowledge of the overall oxylipin profile in order to understand their overall biological effects. Their functions are many, including apoptosis, tissue repair, blood clotting, cell proliferation, blood vessel permeability, pain, inflammation, immune actions and blood pressure regulation (11, 90).

N-6 PUFA Oxylipin Functions

COX oxylipins The most well-known oxylipins are eicosanoids derived from the n-6 PUFA AA. COX derived prostanoids are involved in the regulation of blood pressure, reproduction, diuresis, blood platelet aggregation, modulation of the immune and nervous systems, gastric secretions, cancer, inflammation and the stimulation of smooth muscle contraction, among other effects, as reviewed (10, 12, 106-108). Within these COX metabolites there can be similar and differing effects on these functions. For example, PGI_2 is an anti-aggregatory factor for platelets (109), while TxA_2, serves as a pro-aggregatory factor (110). Another example is the vasodilatory effect of PGI_2 and PGE_2, and the vasoconstrictory effect of $PGF_{2\alpha}$ in some vascular beds (111, 112). PGE_2 also can have effects on thrombosis, which vary depending on the receptor it interacts with. For example, PGE_2 can bind either the EP3 receptor, which makes PGE_2 a pro-thrombotic mediator, or EP4, which makes PGE_2 an anti-thrombotic mediator (113). Similarly, PGD_2 and its metabolites can be both pro-inflammatory and be involved in the resolution of inflammation (34). Compared to COX products formed from AA, those derived from DGLA are usually, but not always less active or produced less efficiently (114). For example, PGE_1 is less stimulatory of aortic smooth muscle cell proliferation than PGE_2 (115). The AdA metabolites, dihomo-PGE_2 and dihomo-PGI_2 also are inactive

or much less active compared to their AA analogues with respect to their platelet aggregating activity and contractile properties in both vascular and nonvascular smooth muscle (80, 116).

LOX oxylipins LOX products such as 5-, 12-, and 15-HETE derived from AA and secreted by epithelial cells and leukocytes are involved in many chronic diseases such as inflammation, obesity, cardiovascular disease, kidney disease and cancer (117-121). As is the case with COX metabolites, AA-derived LOX products can have effects that are both similar to and differing from each other, as well as from those derived via the COX and CYP pathways. For example, 12-HETE has been shown to have both pro- and anti-thrombotic effects (122-124), while TxA_2 is pro-thrombotic (110) and PGI_2 is anti-thrombotic (109). LOX derived HETE and their oxo-ETE metabolites appear to be primarily pro-inflammatory: for example 5-HETE has chemotactic roles in polymorphonuclear leukocytes (PMN) and rabbit alveolar macrophages (125, 126) and stimulates specific granule release from human neutrophils (127). Both 5-oxo-ETE and 12-oxo-ETE also can stimulate eosinophils and neutrophils, but appear to have less activity than their corresponding HETE (128, 129). 5-HETE can also be further converted to 4-series leukotrienes (e.g. LtC_4) that play an important role in inflammation, asthma and allergy (130). Eoxins formed from 15-HpETE also have pro-inflammatory effects (28), and hepoxilins and their metabolites (trioxilins) are another group of oxylipins derived from 12-HpETE that are involved in neutrophil migration and intracellular calcium release (131, 132).

It is important to note, however, that some AA derived oxylipins also display anti-inflammatory and anti-cancer activity. For example, 15-HETE can inhibit degranulation of PMN, superoxide production and endothelial-PMN interaction (133, 134). In addition, 15-HETE can be metabolized to lipoxins, which can be synthesized by epithelial cells and leukocytes and modulate response to injury by mediating apoptosis, resolution of inflammation, and decreasing pain, angiogenesis and cell proliferation (14,

45, 135). Aspirin-triggered lipoxins (e.g. 15-epi-LxA_4) are formed via aspirin acetylated COX2 and 5-LOX and have similar properties to the lipoxins (136, 137).

In addition to AA metabolites, LOX also metabolizes other n-6 PUFA, including LA, GLA, DGLA and AdA. As with AA oxylipins, 9-HODE and 13-HODE derived from LA have been mostly related to pathological conditions such as atherosclerosis, nonalcoholic steato-hepatitis and Alzheimer's disease (138-140), but there are also instances when HODE and their oxo-ODE metabolites are anti-inflammatory and anti-proliferative (141-143). While no functions for GLA oxylipins have been reported, DGLA oxylipins also tend to antagonize the analogous LOX derived AA oxylipins. For example, PGE_1 and 15-HETrE from DGLA have anti-proliferative effects, inhibit cancer cell growth and inhibit bleomycin-induced lung fibrosis (144-146), while 15-HETrE has anti-inflammatory effects in skin (143). Three-series leukotrienes derived from DGLA may also reduce inflammation and broncho-constriction due to their relatively lower production compared to 4-series leukotrienes from AA and possibly lower bioactivity (147, 148).

CYP Oxylipins Oxylipins derived via the CYP pathway from AA include EpETrE and HETE, which have vascular, cardiac and renal functions (13, 149, 150). The effects of these oxylipins also are unique and can be opposing. For example, AA derived EpETrE formed via CYP epoxygenase have hypotensive effects, which is opposite to the hypertensive effects of 20-HETE formed via ω-hydroxylase activity (151, 152). In addition, 16-, 18- and 19-HETE, as well as 20-HETE metabolites (20 COOH AA and 20-OH-PGE_2), also can promote vasodilation (151, 153-155). In some cases, the DiHETrE metabolites of EpETrE formed via sEH activity have less activity (156), but in other cases the DiHETrE have similar or even greater potency (157, 158). Interestingly, sEH inhibitors are currently being used to pharmacologically treat hypertension by prolonging the effects of the epoxy fatty acids on vasodilation (159), but polymorphisms in the CYP enzymes which produce EpETrE do not consistently correlate with effects on

hypertension, as reviewed in (160). In addition, EpETrE also play roles in many other biological functions, such as insulin sensitivity (161), hyperalgesia (94) and tumor angiogenesis and metastasis (162, 163).

CYP oxylipins formed from LA appear to have similar effects to those derived from AA. For example, 9,10- and 12,13-EpOME derived from LA are produced by neutrophils and macrophages, mediating inflammatory effects (164, 165). These oxylipins were originally referred to as leukotoxin and isoleukotoxin, respectively, but later studies indicate that their toxic effects may be due to conversion by sEH to their diol metabolites (166). Elevated EpOME also has been related to extensive burns, respiratory syndrome and a systemic organ failure in burned skin of humans and lung (167).

N-3 PUFA Oxylipin Functions

In general but not always, oxylipins formed from the n-3 PUFA have lesser biological potency when compared to those derived from n-6 PUFA, and often compete for the same receptor, further dampening the biological effect (168). In addition, since they also compete with n-6 PUFA for the same oxylipin biosynthetic enzymes, they may reduce biological activity by reducing the amount of total and n-6 PUFA derived oxylipins produced and increasing the levels of the less active n-3 PUFA derived oxylipins (169, 170).

COX oxylipins. With respect to COX oxylipins, those derived from EPA are similar to DGLA oxylipins, generally being less potent or are produced less efficiently (170) than the analogous oxylipins derived from AA. Hence, PGE_3 compared to PGE_2 binds to the EP4 receptor with less affinity and activity in colorectal cancer cells (168) and demonstrates less mitogenetic and inflammatory activity in fibroblasts and monocytes (168, 171, 172). TxA_3 compared to TxA_2 is produced less efficiently and was reported to have less vasoconstrictory and aggregatory activity (170), but a later study has attributed this reduced biological effect to the presence of PGD_3 in the incubations and found that they have similar aggregatory activities (84). PGI_3 and PGI_2 also have similar

vasodilatory and anti-aggregatory effects on platelets (170) and TxA_2 and TxA_3 have similar ability to elevate plasma catecholamines in rats, or to activate the TP receptor (84, 169, 170, 173).

LOX oxylipins. LOX also metabolizes the n-3 PUFA, ALA to HOTrE, EPA to HEPE and DHA to HDoHE, oxylipins that also tend to have less inflammatory activity or to be anti-inflammatory. There is very little information on ALA derived oxylipins, but recent findings indicate that 9,16-DiHOTrE has anti-inflammatory and anti-aggregatory effects by reducing prostaglandin production (83), and that 9- and 13- HOTrE are associated with reduced glomerular hypertrophy in obese rats (58). An earlier paper indicates that 13-HOTrE may have anti-inflammatory effects in chondrocytes (174), and a recent paper showed that 13-oxo-OTrE can stimulate glucose uptake and differentiation in adipocytes (175). EPA oxylipins have been much more investigated and are primarily anti-inflammatory; for example, 5-HpEPE can be metabolized to LtB_5, which has less activity, and also competes with LtB_4 and therefore reduces inflammation and broncho-constriction (176-178). 5-oxo-eicosapentaenoic acid (5-oxo-EPE) derived from 5-HEPE is 10-fold less potent in stimulating neutrophils compared with the AA oxylipin (5-oxo-ETE) derived from 5-HETE (89). 15-HEPE derived from EPA also exhibits anti-cancer effects. For example, in human prostatic adenocarcinoma cells 15-HEPE can inhibit cancer cell growth and inhibit production of AA oxylipins (179).

DHA also is metabolized via LOX, resulting in the production of HDoHE that also generally exhibit beneficial effects. For example, 4-HDoHE has been reported to inhibit proliferative retinopathy and retinal endothelial cell proliferation (180) and 14-HDoHE can antagonize platelet activation and smooth muscle constriction (181, 182). The functions of 14-HDoHE may be mediated via maresins, as they have been shown to be involved in resolution of inflammation, tissue regeneration and analgesia (97, 183), or via other DiHDoHE which have similar protective effects, such as the wound healing properties of 14,21-DiHDoHE in mice (184) and inhibition of PMN infiltration in a

mouse peritonitis model by 14,20-DiHDoHE (104). Similarly, 17-HDoHE inhibits 5-LOX in rat leukemia cells (85), reduces inflammation and oxidative damage in murine hepatocyte injury (185) and has anti-hyperalgesic properties in a rat model of arthritis (186). Some of these actions may be via the D-series resolvins and protectins derived from 17-HpDOHE. Resolvins have been shown to have protective actions in inflammatory diseases (100, 187, 188), while the effects of protectins vary by isomer – PDX has anti-aggregatory effects (189, 190) and can restore insulin sensitivity in obese mice (191), but PD1 does not exhibit these activities (191, 192). Both can inhibit influenza virus replication (193, 194), reduce inflammation and accelerate the resolution of inflammation (188), with the latter study indicating that PD1 has greater potency in this regard. Helpful reviews delineating differences in structure and functions of the protectins can be found in references (98, 195).

CYP oxylipins. N-3 PUFA oxylipins derived via the CYP pathway also have some similar and some differing effects compared to their n-6 PUFA derived counterparts. EpETE derived from EPA have vasodilatory and anti-inflammatory effects, which is similar to EpETrE derived from AA, with the vasodilatory effects of EpETE possibly exceeding those of EpETrE in some vascular beds (196, 197). In addition, several CYP isoforms preferentially metabolize n-3 over n-6 PUFA, as reviewed in (90, 198). EpETE can also inhibit Ca^{2+} and isoproterenol induced contractility of neonatal cardiomyocytes, suggesting they have antiarrhythmic effects (199). EpDPE derived from DHA has anti-inflammatory, vasodilatory and anti-cancer effects, similar to EpETE (163, 197, 200). EpDPE also can inhibit angiogenesis and metastasis (163), unlike the AA derived EpETrE, which promote these functions (162). 18-HEPE derived from EPA via ω-hydroxylase also appears to have an anti-cancer role by down regulating pro-inflammatory and pro-proliferative factors (201), possibly via conversion to E-series resolvins. These resolvins have similar effects as the D-series resolvins, markedly reducing PMN infiltration, decreasing pro-inflammatory cytokines, and enhancing the

resolution of inflammation (135, 202, 203). More functions were reviewed in (204)

In summary, oxylipins have important biological effects that mediate normal physiology and function. However, compared to oxylipins derived from n-3 PUFA, those derived from n-6 PUFA have more inflammatory, vasoconstrictory, and proliferative effects, with the exception of several examples, such as some prostanoids and/or their metabolites, lipoxins, some oxylipins from DGLA and LA, EpETrE and some CYP derived HETE. On the other hand, most oxylipins derived from n-3 PUFA tend to have less activity or be anti-inflammatory, pro-resolving, vasodilatory, and anti-proliferative. In addition, some of the anti-inflammatory and vasodilatory CYP oxylipins derived from EPA and DHA have even greater potency than their AA counterparts.

1.5 Effect Of Sex On Oxylipins

Sex has been shown to have effects on fatty acid metabolism. For example, after n-3 fatty acid supplement intake, DHA and EPA in red blood cell membranes are significantly higher in females than males (205). Women also have a higher conversion rate of ALA to DHA (206, 207). Also, the free fatty acid flux kinetics is significantly greater in girls than boys, as girls have higher maximum and insulin-suppressible lipolysis rates to create free fatty acids (208). However, there are very few studies that have examined the effect of sex on oxylipin production. It has been shown in a few studies that female rats have higher PGE_2 and TXA_2 levels than males and testosterone decreases PGE_2 production in renal medulla (209). Female mice showed a higher level of LTC_4 and LTB_4 with androgen treatment in peritoneal macrophages (210). However previous research only has examined several individual oxylipins, and there is no study that has investigated the sex effect on oxylipins derived from all PUFA.

1.6 LA Intake Controversy

In 1929, Burr and Burr first identified the function of essential fatty acids (211). LA has several critical functions, which includes producing oxylipins, consisting of tissues, or contributing to skin barrier. However the dietary recommendations around the world varies. Recommendations vary from 1-2% of energy (%E), the level required to prevent fatty acid deficiency, to more than 10%E, the level that is thought to reduce the risk of chronic heart disease. For example, the Institute of Medicine defines an adequate intake of LA as 17g/d for men and 12g/d for women (~5%-6%E) (212). Both the Dietary Reference Intake Report and the 2015-2020 Dietary Guidelines for Americans support 5%-10%E from n-6 PUFA for optimal health (213, 214). The Third Adult Treatment Panel of the National Cholesterol Education Program recommends PUFA intake up to 10%E (215), while the European Commission suggests 4%-8%E (216), the Food and Agriculture Organization/WHO suggests 5%-8%E (217), the British Nutrition Foundation suggests 6.5E% (218), the Department of Health and Ageing Australia and New Zealand suggests 4%-5%E (219) and the American Dietetic Association and Dietitians of Canada suggest 3%-10%E (220). ISSFAL suggests that an adequate LA intake is 2% of energy (221) and the American Heart Association suggests at least 5%-10% of energy should be obtained from n-6 PUFA (222).

The American Heart Association claims that “the consumption of at least 5% to 10% of energy from omega-6 PUFAs reduces the risk of CHD relative to lower intakes” and states “AHA supports an omega-6 PUFA intake of at least 5% to 10%E in the context of other AHA lifestyle and dietary recommendations” (222) . This statement has generated much controversy (223-228). However the conclusions from those reviews are not consistent. A re-analysis of the relationship between coronary heart disease and dietary LA provides strong evidence against the point raised by the AHA. It showed that that

when the oils are made of LA and almost no ALA, they may contribute to a higher risk of death, and that it is difficult to separate benefits of LA and ALA when they are both provided in the diet (229). One of the main questions is whether the oxylipins derived from LA are elevated or whether LA can be converted to AA and AA oxylipins. However there are no data to support this latter concept. When dietary LA is reduced by 90% or increased by 550%, the level of blood AA does not change (230). However our laboratory has shown that tissue fatty acid level does not necessarily represent oxylipin levels (58, 231). There are very few studies that have examined the effect of dietary LA on oxylipin production.

1.7 N-3 Fatty Acid Intake Controversy

Long chain n-3 fatty acids are known to provide many health benefits (232-235). However, a recent meta-analysis has reported that there is no effect of long chain n-3 PUFA on occurrence of sudden death and fatal coronary heart disease (232, 236). But in the earlier trials, they reported that there is a significant reduction in risk for these events (233, 234).

There is still also a debate on how much intake of long chain n-3 fatty acids is enough. Oxylipins derived from EPA and DHA are known to have many beneficial effects; however, how these changes with separate EPA or DHA intake is unknown. In addition to the controversy about the efficacy of long chain n-3 fatty acids, the efficiency of conversion from ALA to DHA and EPA is still not clear and well defined. Vegans can maintain a stable level of EPA and DHA in their plasma (237, 238), which means that ALA can be converted to EPA and DHA for optimal health because vegans do not show a detrimental health status. In addition to this, when measuring the conversion of ALA to DHA by using isotopes, several studies have suggested that ALA can increase the levels

of DHA (239, 240), but at a low rate (0.23% and 0.57%). Similar results that the conversion rate is 0.04%-2.84% were seen in (241, 242). However, some studies have found that ALA intake is not able to increase DHA, but ALA intake can increase the level of EPA (243), which suggests that the conversion from ALA to EPA occurs, and that the conversion from EPA to DHA is poor. On the other hand, feeding purified EPA in humans did not increase DHA (244, 245), and one study showed that in humans the level of DHA was decreased after EPA intake (246). Apparently, there is a controversy over the conversion from ALA to DHA. However no studies have measured the effects of individual n-3 fatty acids on their oxylipin levels.

A study in diseased kidneys showed that without an elevated level of DHA, flax oil feeding can elevate DHA oxylipins (231). This demonstrates that fatty acid data alone are not sufficient to predict the oxylipin profile. Dietary recommendations of ALA are inconsistent around the world, as indicated in Table 1.1. Whether ALA can be converted to DHA oxylipins in normal tissue and the differential effect of ALA, EPA and DHA alone on oxylipin production remains to be elucidated.

To conclude, to date there are no data on the effects of LA, ALA, EPA and DHA on oxylipins in either humans or animals. Oxylipins are the fundamental factors that relate to fatty acid effects on health. However it is unknown how dietary fatty acids alter these fundamental factors with many biological and physiological functions.

Rats are the general model for simulating human tissue and widely used in pharmacology and toxicology studies. Rats are omnivorous, and have similar anatomical and physiological properties to human and they have faster growth and metabolism (247). Therefore, normal rats were used to test the hypotheses outlined in the next section.

Table 1.1 ALA Intake Recommendations.

Organization	Country	LA	ALA	DHA	EPA	DHA+EPA	Reference
ADA USA & Canada (2007)	US and Canada	3-10%E/d	1.1-1.6g/d (1.2-1.5%E/d)	-	-	500mg/d	(248)
FAO/WHO (2008) WHO (2003)	International	2.5-9%E/d	0.5-2%E/d	-	-	0.25-2g/d	(249) (250)
ISSFAL, (1994-2004)	International	2%E/d	0.7%E/d	-	-	>500mg/d	(251)
ADA, (2008)	US	4-8g/d	2g/d	-	-	250mg/d	(252)
NHRC	Australia & New Zealand	8-13g/d	0.8-1.3g/d	-	-	90-160mg/d	(253)
Dietary Reference Intakes for Canada and US	US and Canada	12-17g/d for males; LA 10-12g/d for females	1.2-1.6g/d for males; ALA 1.0-1.1g/day for females	-	-	-	(254)

ADA: American diabetes association; FAO, Food and Agriculture Organization; WHO, World Health Organization; ISSFAL, International Society for the Study of Fatty Acids and Lipids; NHRC, National health and medical research council. If an adult daily calorie intake is 2200, 1%E is calculated almost 0.7g fatty acid.

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Chapter 2 Research Objectives and Hypotheses

2.1 Hypotheses

- The oxylipin profile will be unique for each tissue.
- The rat tissue oxylipin profile will be affected by dietary fatty acid composition.
- Diets with higher LA will increase the LA oxylipin level and the AA oxylipin level.
- Diets with higher levels of individual n-3 fatty acid will increase levels of oxylipins produced from ALA, EPA and DHA.
- There will be sex differences in oxylipins in tissues.

2.2 Objective

The overall objective is to determine the oxylipin profile of and the effect of different types of PUFA on rat tissues. To test these hypotheses, diets with elevated levels of LA, ALA, EPA and DHA will be provided to normal rats. The oxylipin profile will be determined in three representative tissues. The kidney is a rich source of PUFA; the liver is central site of PUFA metabolism; and serum is a readily available material that is the most commonly assessed in human studies, so these tissues will be chosen for analysis.

Chapter 3 Dietary Linoleic Acid (LA) Increases LA and Arachidonic Acid (AA) Derived Oxylipins Despite Not Altering Tissue Fatty Acid Levels

3.1 Introduction

One of the major changes that has occurred in diets worldwide, including the North American diet, is the increase in n-6 fatty acid intake (1). Essentially, this is an increase in linoleic acid (LA), the predominant n-6 fatty acid in the diet. This increase is evidenced by the 136% increase in LA in adipose tissue in the last half century (2).

LA was determined to be an essential fatty acid in 1930 (3), and dietary recommendations for n-6 fatty acid intake since then has focused on defining the optimal level of LA intake. Recommendations vary from 1-2% of energy (%E), the level required to prevent fatty acid deficiency, to more than 10%E, the level that is thought to reduce the risk of chronic heart disease. For example, the Institute of Medicine defines an adequate intake of LA as 17g/d for men and 12g/d for women (~5-6%E) (4). Both the Dietary Reference Intake Report and the 2005 and 2015-2020 Dietary Guidelines for Americans support 5%-10%E from n-6 PUFA for optimal health (5, 6). The Third Adult Treatment Panel of the National Cholesterol Education Program recommends PUFA intake up to 10%E (7), while the European Commission suggests 4%-8% E (8), the Food and Agriculture Organization/WHO suggests 5%-8%E (9), the British Nutrition Foundation suggests 6.5E% (10), the Department of Health and Ageing Australia and New Zealand suggests 4%-5%E (11) and the American Dietetic Association and Dietitians of Canada suggest 3%-10%E (12). ISSFAL suggests that an adequate LA intake is 2% of energy (13) and the American Heart Association suggests at least 5-10% of energy should be obtained from n-6 PUFA (14).

The median LA intake in the US is 11 g for females and 17 g for males, which is approximately 5-6E% of total energy. There is a considerable debate on whether this level of intake of LA is beneficial. From the results of clinical trials, it is concluded that a higher intake of LA is beneficial, as it can help to reduce the risk of chronic heart disease

(14, 15). Others, however, suggest that a high LA intake may not be beneficial, based on its potential to promote pro-inflammatory products derived from AA (i.e. oxylipins) (16, 17). On the other hand, a recent systemic review has shown that the LA-AA pathway is very well regulated. When dietary LA is reduced by 90% or increased by 550%, the level of blood AA does not change. However, there are very few studies that have examined the metabolites derived from fatty acid so it is unclear whether high LA intake will alter AA derived oxylipins, or whether changes in blood adequately reflect tissue levels of these metabolites. Additionally, metabolites formed from LA are rarely examined despite the fact that recent studies have shown that LA oxylipins constitute a major proportion of total tissue oxylipins (18, 19).

After being released by phospholipase A_2, AA is metabolized to oxygenated bioactive lipids (oxylipins) via three pathways: the COX pathway, the LOX pathway and the CYP pathway. LA also is converted by LOX and CYP450 to form oxylipins. The oxylipins derived from AA are the most well- studied and are generally regarded as pro-inflammatory molecules. For example, prostaglandins (PG) and leukotrienes derived from AA have pro-inflammatory properties and promote progression of cancers (20, 21). However, some AA-derived oxylipins also are involved in the resolution of inflammation (22). The oxylipins derived from LA also have differing effects. For example, HODE inhibit proliferation and prevent platelet adhesion (23, 24), while DiHOME cause mitochondrial dysfunction (25), and EpOME have vasoconstrictory effects (26).

There are many studies focused on how diet affects the fatty acid profile of tissues, and conclusions related to health are ascribed based on the fatty acid composition. However, bioactive lipids derived from PUFA are important effectors in biological and physiological process and are usually not measured. Since the oxylipin profile may not necessarily mimic the fatty acid profile (27), it is important to measure both. Also, there are very few studies that have examined the effect of sex on oxylipin production. It has been shown in a few studies that female rats have higher PGE_2 and TXA_2 levels than

males and testosterone decreases PGE_2 production in renal medulla (28). Female mice showed a higher level of LTC_4 and LTB_4 with androgen treatment in peritoneal macrophages (29). However previous research only has examined several individual oxylipins, and there is no study that has investigated the sex effect on oxylipins derived from all PUFA. The purpose of this chapter is to examine whether dietary LA alters oxylipin levels, particularly those derived from AA since they are reported to be resistant to dietary intervention with LA.

3.2 Materials And Methods

3.2.1. Rats And Diet

Six male and six female healthy weanling Sprague-Dawley rats were provided 3 different diets for 6 weeks, for a total of 36 rats. In each diet group, they were provided AIN93G diets except that the diets contained 10g instead of 7g oil/100g diet, and the source of oil varied between diets as outlined below and in the diet composition table (Table 3. 1). This level of fat resulted in diets with 23%E of energy as fat. The 3 diets contained a mixture of oil sources, as outlined in Table 3. 1, resulting in similar saturated and unsaturated fatty acid composition. The adequate LA group has adequate levels of LA and ALA; the high LA group has 3g/100g diet more of LA and the same amount of ALA. The higher LA in the latter diet came primarily at the expense of monounsaturated fatty acid. The high LA+ALA group has soy oil as the sole oil source, as found in the AIN93 diets, resulting in a similar level of LA as the high LA diet and a higher level of ALA than the other diets. As a result, the adequate LA and the high LA+ALA diets had a similar LA/ALA ratio, whereas this ratio was 2.5 times higher in the high LA diet. The fatty acid composition for dietary oils is listed in appendix 1.1; the diet fatty acid composition is calculated based on this. The step by step details for the methodology of preparing diet is located in appendix 2.1, and protocol of the oil fatty acid composition analysis is located in appendix 2.2.

Rats were weighed weekly and terminated after 6 weeks of feeding. Rats were anesthetized with isofluorane and terminated via decapitation to collect trunk blood, which was centrifuged at 800g to obtain serum, and was stored at at -80°C until analysis. The right kidney and a portion of the liver were removed, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. All procedures were performed in accordance with the Canadian Council for Animal Care guidelines and approved by the University of Manitoba Animal Care Committee.

Table 3.1.Diet, Oil And Fatty Acid Composition Of The Diets.

	Adequate LA	High LA	High LA+ALA
		g/100 g diet	
Cornstarch	34.9	34.9	34.9
Casein (87%) (Protein)	20.7	20.7	20.7
Dextrinized cornstarch	13.7	13.7	13.7
Sucrose	10.3	10.3	10.3
Fiber	5.17	5.17	5.17
Mineral mix (AIN93G)	3.62	3.62	3.62
Vitamin mix (AIN 93)	1.03	1.03	1.03
L-Cystine	0.31	0.31	0.31
Choline	0.259	0.259	0.259
TBHQ	0.002	0.002	0.002
Safflower oil	-	4.3	-
Olive oil	7	-	-
Soy oil	2.2	3.8	10
Coconut oil	0.65	1.9	-
Flax oil	0.15	-	-
Total Diet	100	100	100
Fatty acids in the diet		g/100 g diet	
LA	2.13	5.21	5.35
ALA	0.27	0.28	0.71
SFA	1.93	2.37	1.56
UFA	6.63	7.16	8.15
MUFA	4.22	1.66	2.07
PUFA	2.41	5.5	6.08
LA/ALA	7.76	18.31	7.50
n6/n3 Ratio	7.74	18.11	7.45

Cornstarch, Casein (87% protein), Dextrinized cornstarch, Sucrose, Fiber, Mineral mix (AIN93G), Vitamin mix (AIN 93), L-Cystine, Choline bitart, Oilve oil, Soy oil, Coconut oil, Flax oil were purchased from Dyets, Inc, Bethlehem, PA. TBHQ was purchased from Sigma-Aldrich, Inc. The fatty acid composition of the diet is calculated based on appendix 1.1.

3.2.2. Oxylipin Analysis

Kidney and livers were lyophilized and a portion was homogenized in Tyrode's salt solution (pH 7.6). Step by step details for lyophilisation and homogenization is listed in appendix 2.3 and appendix 2.4. Samples for oxylipin analysis were prepared and analyzed by HPLC-MS/MS multiple-reaction monitoring as described (30). Oxylipins scanned but not detected are listed in Appendix 1.2.a. Oxylipins without primary are listed in Appendix 1.3, including their mass transition and expected retention time based on the difference between the experimentally detected retention times of deuterated standards and the published retention times (31). Dose response curves were run to determine detector response factors, which were applied to all oxylipins, unless otherwise noted when primary standards were unavailable, and the oxylipin mass transitions, internal standards, and retention time are listed in appendix 1.4. HPLC solvent gradient is listed in appendix 1.5. Deuterated internal standard used for oxylipin analysis is listed in appendix 1.6. 400µL serum was directly used for oxylipin analysis without homogenization; 200µL of kidney and liver homogenate was used for oxylipin analysis. Briefly after adding 10µL of deuterated internal standards (Cayman Chemical, MI, USA) per 400µL serum and per 200µL kidney and liver homogenate, samples were adjusted to pH<3 by using HCl (Sigma-Aldrich, Inc). Solid phase extraction was with Strata-X SPE columns (Phenomenex, CA, USA) that were preconditioned with methanol and pH3 water, loaded with sample, rinsed with 10% methanol, and eluted with methanol. Samples were dried down and resuspended in solvent for analysis by HPLC/MS/MS (Sciex 6500; Sciex, ON, Canada). Quantification of oxylipins was determined using the stable isotope dilution method (32). Step by step details for solid phase extraction is listed in appendix 2.5.The amount of oxylipins was expressed as ng of oxylipin per g dry tissue in kidney and liver, and ng of oxylipin per mL of serum. Step by step details for oxylipin data analysis from HPLC/MS/MS are listed in appendix 2.6

3.2.3. Fatty Acid Analysis

Aliquots of the kidney and liver homogenates (250µL) as described above were used. For homogenates used for fatty acids, 8.34µL antioxidant cocktail was added immediately after homogenization. After adding 20µL of internal standard [10µL of C150 (10 mg/mL) of phospholipid, 10µL of free fatty acid C170 (2mg/mL) and triacylglyceride C170 (5.5mg/mL) in a 1:1 mixture] per 250µL of tissue homogenate or serum, lipids were extracted via solvent-solvent (2:1 chloroform: methanol with 0.01%BHT) extraction (33). Lipid extracts were purified by thin layer chromatography (TLC) (heptane/isopropyl/acetic acid, 60/40/3, v/v/v) to isolate the phospholipid fraction. After TLC, fatty acids (phospholipid fatty acids for tissues and total fatty acids for serum) were methylated using methanolic HCl at 80°C for 1 hour and quantified by gas chromatography as described in (33). Step by step details for protocol of fatty acid analysis is listed in appendix 2.7. Fatty acids were expressed as µg per g dry tissue for kidney and liver and µg per mL in serum.

3.2.4. Statistical Analysis

Data were analyzed using SAS 9.3 (SAS Institute Inc, Cary, NC). The Shapiro-Wilk test was used to test for normality. Data were analyzed by using two-way analysis of variance (ANOVA) to test the main effects or were analyzed using the Kruskal-Wallis test when data could not be normalized by logarithmic transformation. Outliers were removed if the data could not be normalized and the data point was outside of the mean±3SD. The protected LS Means test was used to detect differences between the 3 diets. All tests were set at a significance level of $P<0.05$. Data are shown as means±SE.

3.3 Results

3.3.1 General Results

All rats grew well throughout the study, with males having higher body weights throughout the study (Table 3. 2). Body weights were not different in rats provided the different diets, although the change in body weight over the length of the study was slightly lower in females given the high LA diet. There were no differences in food intake (data not shown).

The number of oxylipins detected in the kidney, liver and serum ranged from 62-75. The distribution of these oxylipins did not mimic the distribution of fatty acids. For example, in the high LA+ALA group, which has soy oil as the lipid source (as found in the AIN93 diet), liver LA makes up 20-25% of PUFA that are oxylipin precursors, but LA oxylipins make up 50-55% of total oxylipins (Figure 3. 1). The distribution of PUFA that are precursors for oxylipins and the total oxylipins from these PUFA in kidney, liver and serum are provided in Table 3. 3 to provide many other examples of differences in the distribution of fatty acids compared to oxylipins. Heat maps of the relative levels of oxylipin profiles in all 3 tissues are shown in figures 3.2-3.4. The raw data for these heat maps is shown in tables 3.4, 3.6, 3.8. Fatty acid data for 3 tissues are shown in Tables 3.5, 3.7, 3.9.

Table 3.2. Body Weights In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

	Adequate LA		High LA		High LA+ALA		Diet	Sex
	Female	Male	Female	Male	Female	Male		
Week	grams							
0	118±2.95	128±5.11	113±3.05	128±2.83	115±3.43	128±4.92		0.0003
1	174±4.57	205±4.99	163±2.25	201±4.66	167±4.65	197±3.65		<.0001
2	215±6.46	279±4.74	202±2.46	274±7.44	208±5.87	265±5.38		<.0001
3	246±8.04	349±4.57	232±2.73	340±9.72	246±3.24	334±7.11		<.0001
4	280±10.9	415±5	257±3.85	401±12.3	277±2.39	398±6.62		<.0001
5	306±12.1^{A}	465±5.51	280±3.06^{B}	447±12.7	304±3.63^{AB}	445±6.49	0.0411	<.0001
6	318±11.1	510±7.32	289±4.18	493±16.5	322±2.79	494±11.2		<.0001
Gain*	200±9.24^{b}	382±5.62^{a}	176±2.57^{c}	365±15.1^{a}	207±2.95^{b}	365±10.1^{a}	0.0464	

Values are mean±SE.
Differing upper case superscript letters shown on the female values with in a row indicated differences between diets. Differing lower case superscript letters within a row indicate differences between means.
*Gain represents change in body weight over 6 week feeding period.

Figure 3.1. Examples Of The Differences In Oxylipin Compared To Fatty Acid Distributions. Shown is the distribution of oxylipins compared to fatty acids in liver phospholipids of rats provided high LA+ALA diets for 6 weeks. For comparisons for all tissues and diets, see Table 3. 3.

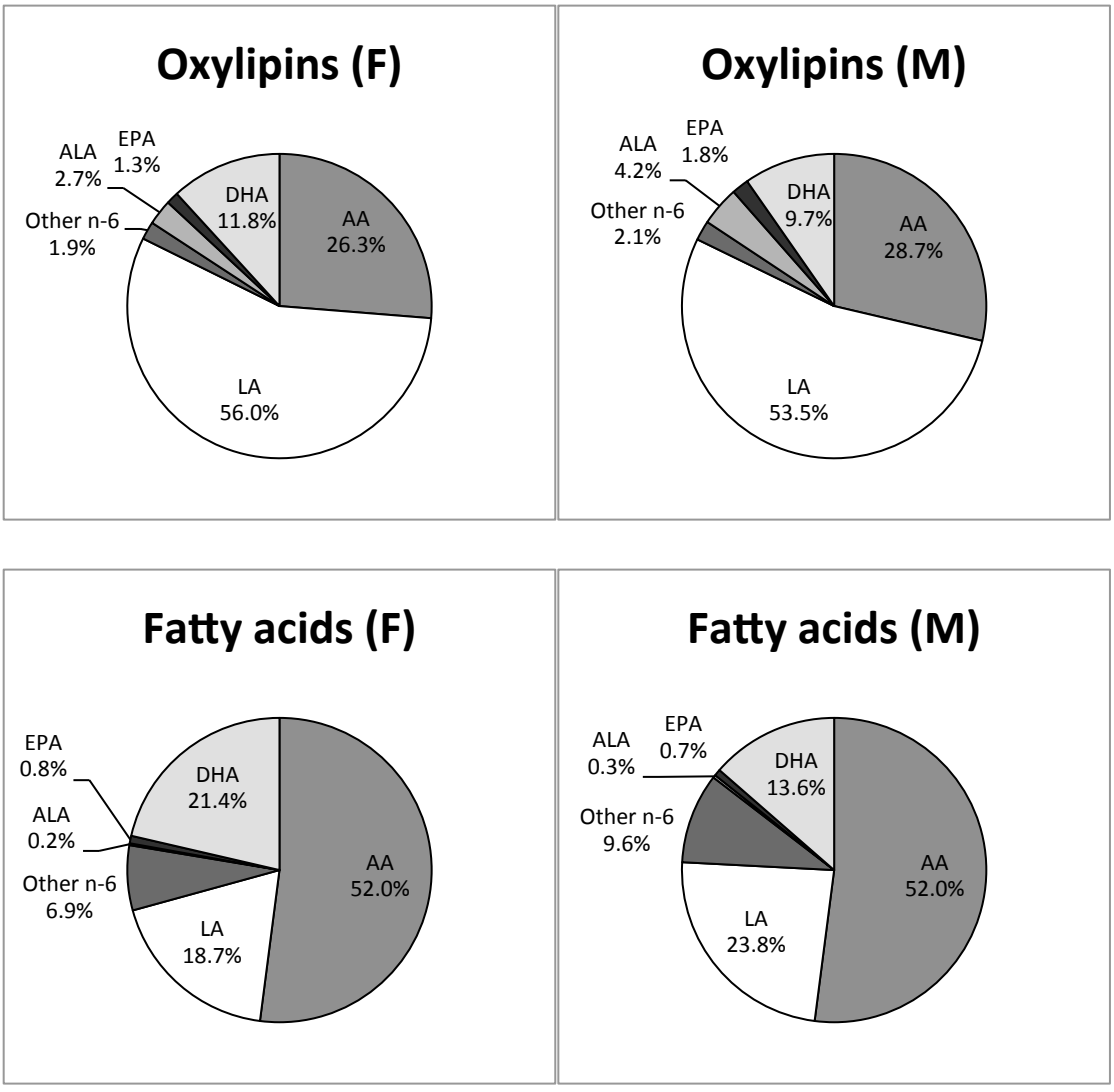


Table 3.3. Differences In Oxylipin Compared To Fatty Acid Mass Distributions For All Tissues And Diets.

				(%)					
Tissue	**Diet**	**Sex**	**FA/Oxylipin**	**AA**	**LA**	**Other n-6**	**ALA**	**EPA**	**DHA**
Kidney	**Adequate LA**	Female	Oxylipin	29.5	48.6	1	0.9	0.8	19.2
			Fatty Acid	66.0	19.1	4.8	0.2	0.6	9.3
		Male	Oxylipin	35.9	48.2	1.4	1.1	1.2	12.3
			Fatty Acid	67.0	22.0	5.8	0.2	0.5	4.7
	High LA	Female	Oxylipin	26.1	61.2	1.1	0.6	0.6	10.4
			Fatty Acid	61.1	25.5	6.5	0.3	0.3	6.4
		Male	Oxylipin	32.3	56.7	1.4	0.8	0.7	8.1
			Fatty Acid	64.4	25.3	6.4	0.2	0.2	3.6
	High LA+ALA	Female	Oxylipin	21.6	59.3	0.9	1.4	0.8	16.0
			Fatty Acid	58.0	28.0	5.1	0.5	0.7	7.8
		Male	Oxylipin	24.5	62.4	1.1	2	0.8	9.3
			Fatty Acid	62.1	28	5.1	0.4	0.4	4.1
Liver	**Adequate LA**	Female	Oxylipin	43.3	33.0	2.6	1.5	1.4	18.2
			Fatty Acid	55.0	13.1	6.9	0.1	0.7	24.1
		Male	Oxylipin	44.1	32.8	3.7	1.4	1.5	16.6
			Fatty Acid	53.7	18.2	10.9	0.2	0.7	16.4
	High LA	Female	Oxylipin	29.8	54.8	2.4	2.2	0.8	10.0
			Fatty Acid	56.6	17.4	7.5	0.1	0.2	18.3
		Male	Oxylipin	32.3	56.1	2.2	1.7	0.6	7.1
			Fatty Acid	56.7	20.9	11.1	0.2	0.3	10.9
	High LA+ALA	Female	Oxylipin	26.3	56.1	1.9	2.7	1.3	11.8
			Fatty Acid	52.1	18.7	6.9	0.2	0.8	21.4
		Male	Oxylipin	28.7	53.6	2.1	4.2	1.8	9.7
			Fatty Acid	52.1	23.8	9.6	0.3	0.7	13.6
Serum	**Adequate LA**	Female	Oxylipin	81.0	11.9	0.4	0.8	1.1	4.9
			Fatty Acid	52.6	32.7	3.4	1.6	0.7	9.1
		Male	Oxylipin	81.5	11.3	0.6	0.7	1.7	4.2
			Fatty Acid	41.7	44.5	4.9	2.7	0.7	5.5
	High LA	Female	Oxylipin	75.3	19.1	0.4	0.6	1.0	3.5
			Fatty Acid	39.0	49.5	4.2	1.5	0.2	5.6
		Male	Oxylipin	75.4	19.4	0.5	0.6	0.8	3.4
			Fatty Acid	37.3	52.3	5.0	1.6	0.1	3.7
	High LA+ALA	Female	Oxylipin	73.2	16.4	0.5	1.8	2.0	6.2
			Fatty Acid	39.6	46.4	2.6	3.1	0.6	7.7
		Male	Oxylipin	76.0	16.0	0.4	1.3	1.9	4.4
			Fatty Acid	36.5	51.6	3.5	3.9	0.4	4.2

Values are % of total oxylipins and of FA that are precursors to oxylipins

Figure 3.2a. Heat Map Of The Kidney N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/g tissue						Interaction	
AA	12-HHTrE								
	PGA_2								
	PGD_2								
	$15deoxy\text{-}PGD_2$							0.0087	
	PGE_2								
	$15k\text{-}PGE_2$							0.0346	
	$PGF_{2\alpha}$								
	$6k\text{-}PGF_{1\alpha}$								
	$11\beta\text{-dhk-}PGF_{2\alpha}$								
	$15deoxy\text{-}PGJ_2$								0.0369
	TXB_2								
	5,15-DiHETE							0.0423	
	8,15-DiHETE								
	5-HETE							0.0235	
	8-HETE							0.0416	
	9-HETE							0.0092	0.0201
	11-HETE								
	12-HETE							0.0113	0.0259
	tetranor 12-HETE							0.003	0.0004
	15-HETE							0.0404	
	HXB_3*								
	LTB_4							0.0078	0.0002
	5-oxoETE								0.0147
	12-oxoETE							0.0032	
	15-oxoETE								0.0278
	5-iso $PGF_{2\alpha}$VI							0.0247	<.0001
	8-iso $PGF_{2\alpha}$III								0.0081
	8-iso 15k $PGF_{2\beta}$							0.0135	0.0002
	5,6-DiHETrE							0.0474	
	8,9-DiHETrE								
	11,12-DiHETrE								
	14,15-DiHETrE								
	16-HETE							0.003	
	18-HETE								
	20-HETE								0.0409
	Sum							0.0044	0.0122
LA	9-HODE							<.0001	
	13-HODE							<.0001	
	9-oxoODE							0.0037	
	13-oxoODE							0.044	0.0408
	9,12,13-TriHOME							0.0232	
	9,10-DiHOME								
	12,13-DiHOME							0.0032	
	Sum							<.0001	
EDA	15-oxoEDE							0.0036	0.036
GLA	13-HOTrE-γ							0.0027	
DGLA	PGE_1								
	$PGF_{1\alpha}$								
	8-HETrE								0.0103
	15-HETrE							0.02	
	Sum							0.04	0.0473
AdA	dihomo $PGF_{2\alpha}$							0.0087	0.0185
	dihomo PGE_2*								
	dihomo PGD_2*								
	Sum n-6							<.0001	
	n6/n3 Ratio							<.0001	<.0001

Legend

Lowest Highest

The P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.
*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

Figure 3.2b. Heat Map Of The Kidney N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/g tissue						Interaction	
ALA	9-HOTrE							<.0001	
	13-HOTrE							<.0001	
	9-oxoOTrE								0.0018
	12,13-DiHODE							<.0001	
	12,13-EpODE							<.0001	
	Sum							<.0001	
EPA	PGE_3							0.0406	
	$PGF_{3\alpha}$								<.0001
	Δ^{17}-6k-$PGF_{1\alpha}$								0.0049
	5-HEPE								0.0091
	9-HEPE							0.0029	
	12-HEPE							<.0001	0.0393
	15-HEPE							0.0005	
	18-HEPE							0.0026	
	Sum							0.0001	
DHA	4-HDoHE							0.0195	
	7-HDoHE							0.0048	
	8-HDoHE							0.0135	0.0001
	10-HDoHE							0.0133	<.0001
	11-HDoHE							0.0006	0.0003
	13-HDoHE							0.021	0.0001
	14-HDoHE							0.0015	0.0181
	16-HDoHE							0.0089	0.0001
	17-HDoHE							0.022	0.0023
	19,20-DiHDoPE							0.0108	<.0001
	20-HDoHE							0.002	<.0001
	Sum							0.0002	<.0001
	Sum n-3							<.0001	0.0002
	n6/n3 Ratio							<.0001	<.0001

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

Figure 3.3a. Heat Map Of The Liver N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/ml						Interaction	
AA	PGD_2								
	PGE_2								
	$15k\text{-}PGE_2$								0.0116
	$PGF_{2\alpha}$								0.0075
	$6k\text{-}PGF_{1\alpha}$								0.0044
	PGJ_2								0.0005
	$15deoxy\text{-}PGJ_2$								
	TXB_2								0.0195
	5-HETE								0.0001
	8-HETE								
	9-HETE								
	11-HETE								0.0182
	12-HETE								
	tetranor 12-HETE								
	15-HETE								0.0055
	5,15-DiHETE								0.0054
	8,15-DiHETE								
	HXB_3								
	LTB_4								0.0044
	$6t,\ 12epi\text{-}LTB_4$								0.0002
	5-oxoETE								
	12-oxoETE								
	15-oxoETE								
	$5\text{-iso } PGF_{2\alpha}VI$							0.0011	
	Sum								0.0446
LA	9,10-EpOME								
	12,13-EpOME							0.0489	
	9,10-DiHOME							<.0001	
	12,13-DiHOME							0.0012	
	9-HODE								0.0288
	13-HODE								
	9-oxoODE							0.0006	
	13-oxoODE							0.0001	
	9,12,13-TriHOME							0.0303	0.0323
	Sum							0.0045	0.0273
GLA	13-HOTrE-γ								
DGLA	PGD_1								0.0301
	$PGF_{1\alpha}$								
	8-HETrE								0.0031
	15-HETrE								0.0004
	Sum								0.0008
	Sum n-6								0.0346
	n6/n3 Ratio							<.0001	

Legend

Lowest Highest

Figure 3.3b. Heat Map Of The Liver N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/g tissue						Interaction	
ALA	9-HOTrE							0.0305	
	13-HOTrE							0.0002	
	9-oxoOTrE							<.0001	
	Sum							0.0124	
EPA	PGE_3							0.0025	<.0001
	5-HEPE								0.0004
	8-HEPE							0.026	
	9-HEPE							0.0146	
	12-HEPE							0.0235	
	15-HEPE								
	18-HEPE								0.0089
	RvE_1								0.0009
	Sum							0.0258	
DHA	20-HDoHE								
	19,20-EpDPE								
	19,20-DiHDoPE								<.0001
	4-HDoHE							0.0179	
	7-HDoHE								
	8-HDoHE								
	10-HDoHE								
	11-HDoHE								
	13-HDoHE								0.0066
	14-HDoHE								
	16-HDoHE								
	17-HDoHE								
	Sum								
	Sum n-6							0.0082	
	Sum n-3								
	n6/n3 Ratio							0.0305	

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

Figure 3.4a. Heat Map Of The Serum N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/ml						Interaction	
AA	PGD_2								
	PGE_2								
	$15k\text{-}PGE_2$								0.0116
	$PGF_{2\alpha}$								0.0075
	$6k\text{-}PGF_{1\alpha}$								0.0044
	PGJ_2								0.0005
	$15deoxy\text{-}PGJ_2$								
	TXB_2								0.0195
	5-HETE								0.0001
	8-HETE								
	9-HETE								
	11-HETE								0.0182
	12-HETE								
	tetranor 12-HETE								
	15-HETE								0.0055
	5,15-DiHETE								0.0054
	8,15-DiHETE								
	HXB_3								
	LTB_4								0.0044
	$6t,\ 12epi\text{-}LTB_4$								0.0002
	5-oxoETE								
	12-oxoETE								
	15-oxoETE								
	$5\text{-iso } PGF_{2\alpha}VI$							0.0011	
	Sum								0.0446
LA	9,10-EpOME								
	12,13-EpOME							0.0489	
	9,10-DiHOME							<.0001	
	12,13-DiHOME							0.0012	
	9-HODE								0.0288
	13-HODE								
	9-oxoODE							0.0006	
	13-oxoODE							0.0001	
	9,12,13-TriHOME							0.0303	0.0323
	Sum							0.0045	0.0273
GLA	13-HOTrE-γ								
DGLA	PGD_1								0.0301
	$PGF_{1\alpha}$								
	8-HETrE								0.0031
	15-HETrE								0.0004
	Sum								0.0008
	Sum n-6								0.0346
	n6/n3 Ratio							<.0001	

Legend

Lowest — Highest

Figure 3.4b. Heat Map Of The Serum N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Adequate LA, High LA, High LA+ALA Diets For Six Weeks.

	Diet	Adequate LA		High LA		High LA+ALA		Effect	
	Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
Fatty Acid		ng/ml						Interaction	
ALA	9-HOTrE							0.0084	
	13-HOTrE							0.0095	
	9-oxoOTrE							0.0228	
	12,13-DiHODE								0.0435
	Sum							0.0097	
EPA	$PGF_{3\alpha}$							0.0088	<.0001
	TXB_3								0.0001
	5-HEPE								0.0002
	8-HEPE							0.0022	
	12-HEPE							0.0266	0.022
	15-HEPE							<.0001	<.0001
	18-HEPE							0.0027	0.0039
	Sum							0.0282	0.025
DHA	4-HDoHE								
	7-HDoHE								
	8-HDoHE								
	10-HDoHE								
	11-HDoHE								
	13-HDoHE								
	14-HDoHE								
	16-HDoHE								
	17-HDoHE							0.0424	
	20-HDoHE								
	19,20-DiHDoPE								
	16,17-EpDPE								
	19,20-EpDPE								
	Sum								
	Sum n-3							0.0181	

Table 3.4a. Kidney N-6 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
			ng/g tissue					
AA derived oxylipins								
12-HHTrE	1487±248	1435±195	1792±203	1388±134	1708±140	1548±156		
PGA_2	140±34.7	142±11.8	164±32.6	162±17.1	164±35	126±33.8		
PGD_2	235±33.1	299±56.9	286±40.1	305±27.2	289±71.7	356±44.7		
15deoxy-PGD_2	32.1±4.2^{B}	45.8±12.5	101±21.9^{A}	96.1±15.1	37.8±17.5^{AB}	83.5±25.5	0.0087	
PGE_2	263±40.8	231±38.9	256±21.4	218±28.3	241±54.3	240±37.1		
15k-PGE_2	13.5±2.52^{c}	28±5.88^{b}	21.2±2.45^{b}	29.2±1.4^{ba}	12.5±1.71^{c}	39.9±5.59^{a}	0.0346	
$PGF_{2\alpha}$	203±21.2	269±33.4	272±54.8	230±32.8	253±48.1	221±32.6		
6k-$PGF_{1\alpha}$	184±19.1	219±35.2	210±32.3	216±20.8	214±74.3	189±20.5		
11β-dhk-$PGF_{2\alpha}$	115±26.0	127±17.1	140±21.0	181±17.1	132±19.3	131±25.6		
15deoxy-PGJ_2	40.2±4.87	55.3±6.92	61.9±13.0	97±10.7	37.8±17.5	61.9±21.0		0.0369
TXB_2	168±36.4	145±17.7	145±5.77	140±11.5	133±27.8	151±10.1		
5,15-DiHETE	152±22.5^{B}	178±17.3	224±33.7^{A}	247±31.5	154±23.5^{BA}	228±27.0	0.0423	
8,15-DiHETE	2493±301	2795±328	2811±253	3160±285	2703±380	3801±534		
5-HETE	3174±303^{d}	4095±233^{abc}	3846±245^{bcd}	5095±376^{a}	3479±422^{cd}	4967±587^{ab}	0.0235❖	
8-HETE	200±24.7^{B}	230±18.0	275±24.5^{A}	301±26.6	274±38.2^{BA}	265±36.7	0.0416	
9-HETE	882±31.3^{B}	1184±78.7	1330±134^{A}	1879±323	1284±132^{A}	1578±239	0.0092	0.0201
11-HETE	1004±141	971±152	1317±73.9	1508±207	1186±152	1181±202		
12-HETE	1196±86.3^{B}	1588±198	1845±252^{A}	3178±743	2116±457^{A}	2964±588	0.0113	0.0259
tetranor 12-HETE	1.92±0.876^{B}	3.97±0.481	3.93±0.598^{A}	7.50±1.28	2.50±0.418^{B}	4.49±0.420	0.003	0.0004
15-HETE	3296±330^{B}	3117±689	4748±242^{A}	5231±645	4071±715^{BA}	4641±934	0.0404	
HXB_3*	0.577±0.172	0.609±0.14	0.804±0.27	1.04±0.189	0.504±0.0864	1.08±0.249		
LTB_4	17.4±3.12^{B}	28.4±4.13	31.2±5.76^{A}	40.6±3.25	17.9±3.44^{B}	35.6±4.10	0.0078	0.0002
5-oxoETE	524±86.6	690±60.9	643±161	1079±245	399±72.7	715±110		0.0147
12-oxoETE	35.0±6.95^{B}	40.1±8.03	63.5±15.8^{A}	93.2±18.7	31.5±5.39^{B}	53.9±14.2	0.0032	
15-oxoETE	275±62.0	421±98.8	396±80.6	632±156	260±59.3	472±101		0.0278
5-iso $PGF_{2\alpha}$VI	202±35.0^{B}	295±21.9	279±35.3^{A}	408±20.0	235.7±47.7B^{A}	394±38.4	0.0247	<.0001
8-iso $PGF_{2\alpha}$III	170±17.9	231±20.5	235±21.1	279±26.6	207±36.8	280±25.8		0.0081
8-iso 15k $PGF_{2\beta}$	14.6±2.04^{B}	28.8±4.09	28.7±8.38^{A}	38.0±2.58	23.0±3.83^{A}	37.2±2.76	0.0135	0.0002
5,6-DiHETrE	56.4±10.8^{ab}	63±12.5^{ab}	53.4±8.79^{ab}	80.9±13.9^{a}	93.9±23.2^{a}	43.6±15.7^{b}	0.0474	
8,9-DiHETrE	19.4±3.40	19.8±3.06	16.2±1.58	21.8±1.87	17.0±2.18	19.3±1.94		
11,12-DiHETrE	57.6±16.6	39.8±4.97	41.9±3.75	41.3±2.96	41.3±4.90	44.4±5.59		
14,15-DiHETrE	39.8±6.02	41.0±4.47	46.7±3.34	57.0±5.87	42.7±2.93	57.3±7.79		
16-HETE	458±43.1^{b}	629±44.3^{ab}	623±49.4^{ab}	845±46.6^{ab}	548±94.1^{ab}	889±175^{a}	0.0030	
18-HETE	14.7±1.69	10.9±1.90	13.2±2.73	14.0±0.400	12.4±2.08	13.4±2.04		
20-HETE	77.3±25.5	37.5±7.73	74.8±24.6	35.3±3.13	70.0±21.0	52.0±9.72		0.0409
Sum	17241±454^{B}	19735±677	22396±1270^{A}	27446±2453	20521±1994^{A}	25369±2847	0.0044	0.0122
LA derived oxylipins								
9-HODE	8525±854^{B}	9166±1551	17446±2051^{A}	18129±1480	19507±3460^{A}	23348±3492	<.0001	

13-HODE	4674±592^{B}	5741±838	11940±1152^{A}	12237±1286	12059±1621^{A}	15816±2780	<.0001	
9-oxoODE	273±21.6^{B}	529±71.9	820±234^{A}	980±178	657±175^{B}	463±110	0.0037	
13-oxoODE	670±85.1^{B}	1426±292	1751±222^{A}	2523±503	1632±461^{A}	2550±737	0.044	0.0408
9,12,13-TriHOME	14013±6206^{B}	9482±878	20325±5860^{AB}	14096±2265	22343±7529^{A}	22026±4010	0.0232	
9,10-DiHOME	114±40.2	64.1±6.19	130±16.4	124±16.4	124±14.1	153±28.7		
12,13-DiHOME	80.8±20.7^{B}	80.5±12.7	105±10.0^{AB}	124±11.8	124±9.58^{A}	165±26.0	0.0032	
Sum	28349±5963^{B}	26489±2472	52517±7923^{A}	48213±4251	56264±8999^{A}	64703±9591	<.0001	
EDA derived oxylipins								
15-oxoEDE	11.9±2.12^{B}	14.8±1.85	25.5±6.30^{A}	34.7±5.39	19.1±4.50^{B}	31.9±5.43	0.0036	0.036
GLA derived oxylipins								
13-HOTrE-γ	78.0±6.05^{B}	102±19.8	187±32.1^{A}	237±54.1	142±19.5^{A}	208±30.3	0.0027	
DGLA derived oxylipins								
PGE_1	26.1±5.58	31.2±3.08	30.8±2.93	36.6±3.71	29.6±5.55	37.5±3.94		
$PGF_{1\alpha}$	40.2±2.22	48.1±4.54	40.0±6.05	48.5±8.23	32.4±4.86	42.0±3.95		
8-HETrE	101±12.6	177±8.94	155±14.2	189±29.4	166±26.4	207±28.3		0.0103
15-HETrE	276±23.2^{B}	326±27.5	435±37.1^{A}	563±116	407±83.6^{A}	551±113	0.02	
Sum	438±39.5^{B}	582±27.2	661±53.1^{A}	837±152	635±118^{A}	838±142	0.04	0.0473
AdA derived oxylipins								
dihomo $PGF_{2\alpha}$	55.9±15.6^{B}	66.7±9.21	86.3±12.1^{A}	108±14.3	53.9±11.4^{BA}	81.3±6.42	0.0087	0.0185
dihomo PGE_2*	0.0504±0.0242	0.0495±0.0145	0.0561±0.00251	0.0729±0.0129	0.0359±0.00799	0.0578±0.0117		
dihomo PGD_2*	0.0225±0.001	0.0405±0.00677	0.0447±0.00232	0.06±0.00901	0.0348±0.00842	0.133±0.0844		
Sum n-6	46174±6112^{B}	46990±2846	75873±8213^{A}	76876±4833	77635±10870^{A}	91232±12054	<.0001	
n6/n3 Ratio	3.92±0.452^{B}	6.27±0.611	7.90±0.820^{A}	9.97±0.776	4.58±0.612^{B}	7.68±0.785	<.0001	<.0001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.4b. Kidney N-3 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
			ng/g tissue					
ALA derived oxylipins								
9-HOTrE	347±62.8^{B}	347±42.2	289±58.3^{B}	345±59.5	719±90.4^{A}	1111±282	<.0001	
13-HOTrE	156±13.0^{B}	192±26.7	206±50.6^{B}	245±49.5	507±73.8^{A}	739±166	<.0001	
9-oxoOTrE	32.5±3.04	70.9±11.2	47.8±7.15	84.9±22.1	43.5±8.58	151±51.6		0.0018
12,13-DiHODE	7.38±1.85^{B}	8.33±2.13	5.54±1.10^{C}	2.99±0.811	14.3±0.918^{A}	21.5±4.31	<.0001	
12,13-EpODE	58.2±6.4^{B}	67.2±9.13	69±13.8^{B}	69.4±12.7	156±14.3^{A}	205±37.9	<.0001	
Sum	542±73.4^{B}	618±72.1	548±105^{B}	677±106	1284±153^{A}	2022±444	<.0001	
EPA derived oxylipins								
PGE_3	23.1±4.28^{AB}	24.6±4.01	18.0±2.53^{B}	22.3±2.14	27.2±4.80^{A}	33.5±4.47	0.0406	
$PGF_{3\alpha}$	20.1±5.37	6.49±0.81	23.7±5.16	4.75±0.69	19.5±4.30	7.03±0.998		<.0001
Δ^{17}-6k-$PGF_{1\alpha}$	1.88±0.836	0.87±0.324	1.14±0.296	0.191±0.127	1.39±0.258	0.409±0.247		0.0049
5-HEPE	144±11.8	251±30.0	159±20.2	263±79.5	230±33.7	288±36.6		0.0091
9-HEPE	48.1±11.6^{B}	60.2±7.77	37.5±8.45^{B}	52.6±8.54	85.1±8.83^{A}	76.8±12.0	0.0029	
12-HEPE	51.5±5.85^{B}	75.1±9.28	50.6±7.25^{B}	70.4±14.8	114±12.5^{A}	145±22.3	<.0001	0.0393
15-HEPE	50.8±4.06^{B}	65.6±6.16	50.7±3.89^{B}	67.7±10.9	93.8±9.9^{A}	111±19.1	0.0005	
18-HEPE	138±19.5^{B}	146±30.7	136±8.72^{B}	96.4±13.3	230±35.4^{A}	209±39.3	0.0026	
Sum	477±15.3^{B}	630±64.9	477±39.5^{B}	577±68.4	801±64.6^{A}	871±98.9	0.0001	
DHA derived oxylipins								
4-HDoHE	2065±235.97^{BA}	1834±219	1572±82.3^{B}	1599±207	2733±410^{A}	2357±500	0.0195	
7-HDoHE	274±28.4^{ab}	155±7.88^{b}	206±5.79^{ab}	230±71.1^{b}	374±54.8^{a}	202±15.7^{ab}	0.0048❖	
8-HDoHE	465±31.3^{B}	270±31.3	341±26.6^{B}	309±28.9	591±90.1^{A}	353±34.0	0.0135	0.0001
10-HDoHE	396±65.5^{B}	181±16.0	295±30.0^{B}	193±26.5	532±94.6^{A}	271±41.1	0.0133	<.0001
11-HDoHE	514±52.2^{B}	324±25.7	393±20.4^{B}	327±25.1	723±85.4^{A}	445±65.5	0.0006	0.0003
13-HDoHE	836.14±141.53^{BA}	394±50.9	622±62.8^{B}	425±73.0	1048±151^{A}	591±97.0	0.021	0.0001
14-HDoHE	1150±129^{B}	692±107	917±91.0^{B}	838±135	1721±201^{A}	1239±239	0.0015	0.0181
16-HDoHE	563±85.8^{B}	275±27.9	456±35.7^{B}	364±69.5	769±84.4^{A}	439±68.2	0.0089	0.0001
17-HDoHE	1834±308^{B}	781±189	1609±207^{B}	1403±351	2779±278^{A}	1550±381	0.022	0.0023
19,20-DiHDoPE	72.0±18.2^{A}	26.6±4.17	33.4±4.79^{B}	20.0±2.1	55.9±5.47^{A}	27.5±1.60	0.0108	<.0001
20-HDoHE	3019±257^{B}	1798±185	2449±430^{B}	1467±195	3854±218^{A}	2104±232	0.002	<.0001
Sum	11194±1021^{B}	6734±686	8902±705^{B}	6839±715	15199±1235^{A}	9591±1288	0.0002	<.0001
Sum n-3	12213±1003^{B}	7981±663	9927±721^{B}	8093±718	17284±1111^{A}	12483±1604	<.0001	0.0002

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.5. Kidney Phospholipid Fatty Acids In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
				ug/g tissue				
C140	104±8.91^{B}	136±13.6	151±10.9^{A}	163±25.1	79±4.96^{B}	122±14.7	0.0024	0.0245
C160	18378±1377^{A}	15823±804	14683±853^{B}	14126±704	15643±862^{AB}	16548±533	0.0202	
C161	527±104	618±125	425±53.0	438±88.5	598±74.2	520±65.6		
C170	140±23.3	224±103	197±31.5	340±134	210±29.7	195±20.1		
C180	16288±1205	15098±1269	13203±928	14132±877	13543±1134	15856±1044		
C181	10034±249^{A}	6891±689	5095±363^{B}	3930±166	5034±333^{B}	4478±225	<.0001	<.0001
C182n6	7803±501^{B}	8268±638	8597±527^{B}	9100±538	9996±745^{A}	11798±535	0.0002	
C183n3	99.2±8.8^{B}	63.6±12.1	89.4±9.28^{B}	60.4±10.5	171±16.1^{A}	152±11.6	<.0001	0.0078
C183n6	62.7±4.69^{ab}	41.5±10.0^{b}	79.9±6.50^{a}	62.8±16.6^{ab}	64.3±10.4^{ab}	90.0±8.38^{a}	0.0467❖	
C200	345±27.9^{a}	210±10.8^{c}	270±18.1^{b}	200±14^{c}	256±26.1^{cb}	228.67±15.46^{cb}	0.0348	
C201	193±15.3^{a}	138±10.3^{b}	88.5±9.48^{c}	90.4±9.56^{c}	93.6±19.8^{c}	100±6.15^{c}	0.0385	
C202n6	180±9.81^{B}	146±12.4	333±20.5^{A}	283±22.0	300±35.5^{A}	320±31.8	<.0001	
C203n3	65.9±22.9	72±15.3	96.4±6.46	71.4±10.4	92.6±21.8	119±16.9		
C203n6	694±59.6	1057±102	614±54.2	789±67.1	690±79.2	865±61.4		0.0005
C204n6	27022±2088	25223±2181	20579±1504	23170±1455	20700±1559	26161±1290		
C205n3	229±23.7^{A}	173±20.1	88.7±8.08^{B}	75.2±4.75	245±19.3^{A}	168±4.30	<.0001	0.0022
C220	840±89.4	465±24.8	659±59.7	465±29.4	692±48.7	531±28.6	0.2352	<.0001
C221	55.7±10.8	28.8±4.38	12.9±6.14	23.4±11.9	24.6±6.31	29.4±9.55		
C222n6	93.3±42.2	111±40.6	107±44.0	167±41.2	149±35.3	129±39.2		
C224n6	637±51.2	550±45.4	655±50.3	625±34.6	506±44.4	610±22.4		
C225n3	242±26.8^{AB}	209±21.0	212±35.7^{B}	167±13.9	258±35.6^{A}	282±33.0	0.0303	
C225n6	302±45.5^{B}	259±46.9	402±34.6^{A}	390±39.0	103±21.8^{C}	125±14.2	<.0001	
C226n3	3800±352^{A}	1777±188	2156±218^{B}	1279±61.6	2774±211^{A}	1712±122	0.0001	<.0001
C240	3979±462	3253±224	3427±381	3396±257	3698±295	3728±208		
C241	1655±117^{A}	1421±79.0	800±34.9^{B}	829±69.9	921±58.9^{B}	981±50.6	<.0001	
n6/n3 Ratio	8.59±0.246^{C}	16.2±0.545	12.7±0.897^{A}	22±0.745	9.73±0.493^{B}	16.9±0.293	<.0001	<.0001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.6a. Liver N-6 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
			ng/g tissue					
AA derived oxylipins								
PGA_2	33.4±10.4^{bc}	20.2±13.1^{c}	82.4±15.0^{a}	33.1±8.13^{bc}	33.5±3.66^{bc}	58.2±13.0^{ba}	0.013❖	
PGD_2	354±84.2^{AB}	533±71.3	514±105^{A}	471±43.1	244±36.8^{B}	399±46.9	0.0239	0.0181
15deoxy-PGD_2	21.4±6.47	34.0±3.92	26.3±5.31	45.8±8.03	13.8±2.87	31.4±3.77		0.0007
PGE_2	166±34.4	247±29.5	211±34.6	239±16.2	121±15.8	226±34.8		0.0051
bicyclo PGE_2	10.7±3.98^{B}	10.6±4.25	65.8±20.1^{A}	41.3±11.4	42.6±11.8^{A}	56.8±27.7	0.001	
6k-$PGF_{1\alpha}$	8.04±0.73^{B}	9±0.403	9.50±1.41^{A}	15.4±1.04	6.19±1.04^{A}	11.8±1.85	0.0038	0.0001
$PGF_{2\alpha}$	242±49.9	186±15.4	260±13.6	203±21.2	166±22.5	168±18.1		
15deoxy-PGJ_2	43.4±11.3^{B}	17.8±10.9	91.8±12.7^{A}	84.7±9.55	51.3±10.4^{A}	80.6±13.0	0.0001	
TXB_2	27.2±3.76	36.8±4.77	32±3.02	42.4±5.23	22.4±3.57	35.1±5.19		0.0051
8,15-DiHETE	494±133	731±83.7	828±268	985±179	550±80.3	880±240		
5-HETE	1021±300	1377±176	1401±322	1825±246	786±95.3	1489±329		0.0089
8-HETE	177±48.5^{B}	151±24.1	287±59.2^{A}	235±39.3	103±12.8^{B}	199±47.4	0.0167	
9-HETE	636.8±200^{AB}	746±126	1031±209^{A}	948±156	434±54.3^{B}	797±198	0.0466	
11-HETE	461±158	457±81.2	644±135	428±100	315±117	425±133		
12-HETE	3891±1122	2892±614	3689±1663	3072±657	2517±350	3666±1104		
tetranor 12-HETE	11.6±2.33	3.24±0.84	23.5±8.22	7.89±2.38	12.0±3.04	7.93±2.67		0.0076
15-HETE	1795±503	2471±602	1737±357	2189±243	1657±212	2372±419		
LTB_4	–$^{\#b}$	–^{b}	7.62±1.85^{a}	7.19±1.02^{a}	6.20±0.99^{a}	7.12±1.28^{a}	0.0002	
5-oxoETE	135±33.9	87.8±9.63	179±44.5	205±49.4	129±25.6	143±34.4		
12-oxoETE	24.6±4.85^{B}	16.8±2.24	39.0±5.48^{A}	30.6±5.08	27.3±3.33^{B}	20.7±4.07	0.0073	0.0275
15-oxoETE	40.9±12.8^{B}	30.3±4.64	61.4±11.9^{A}	63.0±10.6	42.3±8.55^{B}	38.0±6.72	0.0109	
20 COOH AA*	0.461±0.0981	0.450±0.121	0.765±0.116	0.419±0.0605	0.888±0.248	0.412±0.0955		0.0187
5,6-DiHETrE	87.7±31.8	199±41.4	75.8±8.77	319±64.2	46.4±5.81	197±42.2		<.0001
8,9-DiHETrE	35.2±7.41	289±47.9	50.6±10.5	416±127	30.3±2.66	261±41.2		<.0001
11,12-DiHETrE	115±23.9	831±144	172±44.8	1053±296	102±9.58	698±119		<.0001
14,15-DiHETrE	101±22.5	917±198	157±20.6	1170±329	96.2±11.6	735±145		<.0001
8,9-EpETrE	3.89±2.13	7.38±1.94	7.4.0±2.51	5.62±1.28	4.78±0.37	6.34±0.52		
16-HETE	3689±534	3080±390	4138±1038	3542±682	3909±875	3768±876		
17-HETE	54.5±4.44	52.9±7.71	52.3±11.8	53.9±8.83	50.8±11.2	68.7±13.2		
18-HETE	22.4±5.63	46.7±11.7	61.4±22.3	48.7±18.5	18.4±5.90	34.2±10.6		
19-HETE	115±22.9	430±89.7	230±82.3	427±108	138±11.3	363±73.7		0.0003
20-HETE	45.5±9.54^{B}	55.1±10.8	106±33.2^{A}	82.3±20.4	63.5±10.9^{B}	45.2±9.41	0.0415	
5-iso $PGF_{2\alpha}$VI	37.8±10.1^{B}	48.1±5.36	53.8±12.3^{A}	95.4±15.8	31.7±5.8^{B}	61.1±15.8	0.0205	0.008
8-iso $PGF_{2\alpha}$III	28.2±6.31^{B}	34±4.04	43.5±7.69^{A}	59.6±9.41	26±3.22^{B}	43.4±9.64	0.0174	0.0329
Sum	13927±2852	16042±2221	16321±2904	18436±1638	11140±791	17384±3183		
LA derived oxylipins								
9-HODE	3698±1008^{B}	5420±1070	10408±2804^{A}	11221±2733	6130±1091^{A}	12016±3039	0.0013	0.05
13-HODE	1610±452^{B}	2651±647	5192±1413^{A}	4599±1176	2630±457^{A}	4989±1245	0.0028	

9-oxoODE	89.8±24.2^{B}	45.1±6.57	202±41.4^{A}	339±89.5	175±41.4^{A}	194±41.4	<.0001	
13-oxoODE	101±33.7^{B}	83.9±16.4	448±114^{A}	490±84.9	312±86.7^{A}	336±25.9	<.0001	
9,12,13-TriHOME	4570±1086^{B}	2017±357	12199±4065^{A}	12357±4839	13450±3873^{A}	12560±5221	0.0005	
9,10-DiHOME	348±103^{B}	931±145	1111±372^{A}	1767±402	755±84.3^{A}	1412±159	0.0006	0.0001
12,13-DiHOME	159±37.7^{B}	705±145	317±45.4^{A}	1237±309	231±23.9^{A}	935±123	0.0077	<.0001
9,10-EpOME	28.7±8.68	35.8±9.6	76.8±26.1	48.3±20.1	42.4±4.61	39.7±7.52		
12,13-EpOME	23±7.1	28.5±7.26	41.9±18	42.8±14.2	27.2±5.51	39.5±5.82		
Sum	10628±2199^{B}	11917±1970	29995±7348^{A}	32095±8676	23754±5297^{A}	32521±9391	0.0001	
GLA derived oxylipins								
13-HOTrE-γ	77.2±20.3^{b}	94.4±16.3^{b}	263±64.9^{a}	121±8.86^{b}	87.0±13.6^{ab}	167±43.4^{b}	0.0128	
EDA derived oxylipins								
15-oxoEDE	1.96±0.569^{B}	2.51±0.597	10.7±2.67^{A}	18.9±5.74	6.57±1.19^{A}	14.3±4.27	<.0001	0.0338
DGLA derived oxylipins								
$PGF_{1\alpha}$	16.3±4.89	15.4±3.47	23.9±2.31	9.45±3.09	11.4±3.68	5.87±3.77		0.0259
5-HETrE	41.5±8.83^{A}	149±21.8	39.6±12.7^{B}	77.4±13.1	13.6±1.44^{C}	45.9±7.49	<.0001	<.0001
8-HETrE	36.0±9.29	96.1±27.4	57.2±9.61	62.9±8.83	32.2±5.24	55.8±14.6		0.0169
15-HETrE	647±128	958±243	808±233	887±172	607±141	927±285		
Sum	741±145	1218±280	928±255	1036±175	665±145	1035±299		
AdA derived oxylipins								
dihomo PGD_2*	0.00238±0.00238^{C}	0.00797±0.00509	0.0529±0.0121^{A}	0.0526±0.00622	0.0151±0.00285^{B}	0.0278±0.00792	<.0001	
dihomo $PGF_{2\alpha}$	24.7±3.97^{B}	22.6±4.77	101±15^{A}	84.7±13.3	28.4±2.94^{B}	44.5±11.7	<.0001	
Sum n-6	25400±4907^{B}	29296±4219	47619±10403^{A}	51772±9751	35679±5623^{A}	51166±12673	0.0082	
n6/n3 Ratio	4.14±0.397^{d}	4.21±0.151^{cd}	6.62±0.49^{b}	9.73±0.662^{a}	5.32±0.592^{c}	5.23±0.274^{c}	0.0305	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
# Denotes that oxylipin is not detected.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.6b. Liver N-3 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
				ng/g tissue				
ALA derived oxylipins								
9-HOTrE	408±116^{B}	423±97.9	1030.67±378.49^{AB}	809±320	974±265^{A}	2144±946	0.0305	
13-HOTrE	61.6±16.8^{B}	68.8±12.5	167±41.8^{A}	137±45.4	159±41.4^{A}	354±136	0.0002	
9-oxoOTrE	11.7±3.27^{B}	4.06±0.4	21.1±3.72^{A}	26.6±6.57	28.3±4.69^{A}	38.1±12.8	<.0001	
Sum	481±134^{B}	496±110	1218±419^{AB}	973±371	1156±297^{A}	2536±1090	0.0124	
EPA derived oxylipins								
PGE_3	19.4±2.76^{A}	54.8±7.41	14.8±1.01^{B}	31.1±3.75	24.9±5.09^{A}	73.4±12.3	0.0025	<.0001
5-HEPE	38.9±9.68	98.6±28.1	46.5±16.8	57.7±6.8	51±9.11	110±15.8		0.0004
8-HEPE	12.7±2.91^{b}	16.4±2.17^{b}	16.2±2.56^{b}	16.6±2.55^{b}	16.4±3^{b}	34.5±7.01^{a}	0.0260❖	
9-HEPE	21.8±4.9^{b}	35.5±7.08^{ba}	33.9±7.97^{ba}	21.3±3.38^{b}	22.8±4.48^{b}	59.2±17.1^{a}	0.0146	
12-HEPE	196±50.6^{AB}	165±33.2	200±87.1^{B}	111±27.8	248±62^{A}	403±129	0.0235	
15-HEPE	110±28.5	94.9±11.9	94.3±34.3	81.3±18.8	132±46.4	278±106		
18-HEPE	35.5±10.4	73.6±22.1	38.5±11.7	34.5±5.89	38.4±4.61	96.0±17.0		0.0089
RvE_1	6.14±1.09	13.3±2.84	4.98±1.92	11.8±2.35	6.53±1.63	7.88±1.3		0.0009
Sum	441±105^{AB}	552±106	446±155^{B}	365±65	540±127^{A}	1062±291	0.0258	
DHA derived oxylipins								
20-HDoHE	928±291	1198±211	1000±346	865±128	796±72.9	1119±189		
19,20-EpDPE	18.2±4.88	21.8±4.76	13.8±5.38	10.2±1.91	9.63±1.61	13.7±2.96		
19,20-DiHDoPE	110±25.1	346±50.8	87.3±9.23	220±26.6	91.4±10.6	263±46.8		<.0001
4-HDoHE	668±171^{A}	1005±255	475±109^{B}	363±104	421±71.8^{B}	503±123	0.0179	
7-HDoHE	184±60.6	150±11.6	226±74.1	179±59.6	133±17.2	180±37.5		
8-HDoHE	270±82.8	271±31.6	273±76.0	235±42.1	188±18.5	264±46.2		
10-HDoHE	136±35.1	130±13.4	187±39.8	106±18.9	142±42.1	136±26.0		
11-HDoHE	271±74.5	326±47.3	290±79.2	215±40.8	206±46.0	247±49.2		
13-HDoHE	754±175	520±89.3	697±169	319±70.6	605±68.8	477±93.9		0.0066
14-HDoHE	1327±407	879±141	728±119	593±126	912±89.4	977±198		
16-HDoHE	249±68.1	219±30.4	296±91.0	175±34.7	283±49.9	290±42.1		
17-HDoHE	944±259	963±119	1349±355	786±165	1189±170	1431±260		
Sum	5859±1534	6027±936	5501±1186	4066±711	4976±444	5900±827		
Sum n-6	25400±4907^{B}	29296±4219	47619±10403^{A}	51772±9751	35679±5623^{A}	51166±12673	0.0082	
Sum n-3	6781±1732	7075±1106	7165±1510	5403±1098	6673±592	9498±1902		
n6/n3 Ratio	4.14±0.397^{d}	4.21±0.151^{cd}	6.62±0.49^{b}	9.73±0.662^{a}	5.32±0.592^{c}	5.23±0.274^{c}	0.0305	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.7. Liver Phospholipid Fatty Acids In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
				ug/g tissue				
C140	68.4±16.8	134±9.53	98.2±10.3	164±29.8	61.5±2.76	141±15.2		<.0001
C160	7698±527	9709±625	8131±514	11129±726	7954±257	10863±479		<.0001
C161	310±30.9^{b}	466±101^{a}	180±14.1^{b}	576±45.9^{a}	207±24.4^{b}	572±30.2^{a}	0.0469❖	
C170	113±22.2^{B}	69.7±13.2	187±17.2^{A}	108±18.2	1234±11.4^{AB}	110±13.7	0.0074	0.0019
C180	20612±4601	15148±1707	24802±1828	14828±1187	24143±1661	11795±1037		<.0001
C181	1928±57.5^{A}	2678±181	1233±94.5^{B}	1794±127	1185±77.6^{B}	1788±81.8	<.0001	<.0001
C182n6	4435±411^{B}	5147±549	6930±511^{A}	7650±799	6701±406^{A}	7643±580	<.0001	0.093
C183n3	45.9±20.4	48.3±18.1	46.7±17.6	66.5±15.9	74.1±15.1	105±19.7		
C183n6	39±9.39	129±21.6	87.1±30.4	183±27.8	66.4±11	158±15.1		<.0001
C200	71.3±7.94^{B}	62.1±7.61	95.8±4.86^{A}	88.6±15.4	75.9±7.96^{AB}	70.5±9.3	0.0454	
C201	90.6±10.4	111±8.1	82±4.14	110±16.5	71.5±7.97	132±15.6		0.0006
C202n6	166±29.1^{B}	155±12.2	438±30^{A}	469±77.4	316±15.4^{A}	439±59.4	<.0001	
C203n3	50.3±18^{B}	18.3±7.73	24.8±1.29^{B}	26.8±4.97	62.7±6.97^{A}	69.2±12.2	0.0002	
C203n6	651±44.1	1501±207	690±175	1279±112	869±140	1588±122		<.0001
C204n6	18705±726^{B}	15177±635	22598±1918^{A}	20745±1695	18717±971^{B}	16745±1115	0.0019	0.0136
C205n3	251±65.5^{A}	190±39	61.9±7.96^{B}	94.1±30.1	273±36.1^{A}	214±22.6	<.0001	
C220	351±57.9	290±39.9	363±52.7	284±49.2	360±27.7	318±43.6		
C221	34.9±19.8	205±84	67.6±58.6	140±76.8	33.2±19.8	196±46.9		0.0068
C222n6	326±198	246±120	406±246	31.4±17.1	465±195	51.6±39.4		0.0348
C224n6	300±55.7^{bc}	260±18^{bc}	446±90.2^{ab}	545±93.7^{a}	240±36.4^{c}	351±58.6^{bc}	0.0087	
C225n3	198±33^{B}	364±51.5	320.2±62.7^{AB}	423±72.7	386±55.1^{A}	608±79.3	0.003	0.001
C225n6	871±136^{B}	799±69.5	938±90^{A}	1547±200	502±35.3^{C}	488±101	<.0001	
C226n3	8199±938	4638±257	7296±746	3986±377	7683±555	4377±428		<.0001
C240	808±100	411±19.9	872±60.2	537±55.4	791±48.9	475±36.4		<.0001
C241	521±63.9^{A}	259±14.4	341±31.2^{B}	227±18.5	277±32.2^{C}	164±10.1	<.0001	<.0001
n6/n3 Ratio	3.28±0.072^{d}	4.55±0.08^{bc}	4.32±0.264^{c}	6.82±0.532^{a}	3.34±0.093^{d}	5.31±0.252^{b}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 3.8a. Serum N-6 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	Female	Male	Female	Male	Female	Male	Diet	Sex
				ng/ml				
AA derived oxylipins								
PGD_2	2.18±0.589	3.34±0.802	2.48±0.741	3.13±1.02	1.42±0.275	2.67±0.729		
PGE_2	3.64±0.963	5.01±1.10	4.11±1.25	5.48±1.88	2.79±0.566	4.87±1.29		
15k-PGE_2	0.0232±0.00723	0.0821±0.0321	0.0193±0.00406	0.0593±0.0196	0.0284±0.0133	0.0861±0.0404		0.0116
$PGF_{2\alpha}$	1.19±0.290	1.92±0.336	1.41±0.324	2.12±0.545	0.971±0.140	2.12±0.468		0.0075
6k-$PGF_{1\alpha}$	0.0865±0.0236	0.108±0.0154	0.0501±0.00603	0.0983±0.0245	0.0568±0.0147	0.0842±0.0141		0.0044
PGJ_2	0.63±0.163	2.85±1.25	0.592±0.173	1.57±0.628	0.363±0.130	1.62±0.431		0.0005
15deoxy-PGJ_2	0.252±0.046	0.285±0.0562	0.200±0.0723	0.287±0.109	0.0989±0.00675	0.256±0.0699		
TXB_2	29.7±9.9	48.9±7.73	30.2±7.46	41.6±11.1	21.2±3.38	44.6±11.3		0.0195
5-HETE	2.09±0.169	3.62±0.651	2.43±0.299	4.03±0.649	2.11±0.219	3.91±0.795		0.0001
8-HETE	0.504±0.0913	0.709±0.094	0.618±0.0901	0.681±0.0947	0.43±0.081	0.665±0.154		
9-HETE	1.19±0.148	1.70±0.289	1.39±0.214	1.73±0.251	1.14±0.182	1.71±0.534		
11-HETE	8.07±2.18	15.5±3.61	8.27±1.67	9.71±2.14	5.54±0.985	11.4±3.06		0.0182
12-HETE	121±30.9	175±29	139±25.5	146±24.1	103±15.8	153±34.7		
tetranor 12-HETE	0.141±0.0384	0.128±0.0197	0.179±0.045	0.165±0.0234	0.125±0.0204	0.188±0.026		
15-HETE	7.77±1.80	15.3±3.55	8.08±1.62	11.1±2.29	6.40±1.04	12.0±2.89		0.0055
5,15-DiHETE	0.0241±0.00361	0.036±0.00805	0.0209±0.00542	0.043±0.00738	0.0313±0.00457	0.0636±0.0195		0.0054
8,15-DiHETE	–[#]	0.0962±0.0962	–	–	–	–		
HXB_3	0.694±0.161	1.09±0.184	0.982±0.161	1.09±0.174	0.873±0.105	1.1±0.252		
LTB_4	0.0255±0.00455	0.0452±0.00863	0.0300±0.00793	0.0549±0.0152	0.0295±0.00686	0.0501±0.0129		0.0044
6t, 12epi-LTB_4	0.226±0.0352	0.570±0.130	0.238±0.0462	0.481±0.144	0.205±0.0354	0.379±0.0741		0.0002
5-oxoETE	0.826±0.0808	1.07±0.215	1.36±0.275	1.68±0.409	0.892±0.130	1.68±0.541		
12-oxoETE	1.80±0.618	0.900±0.129	1.34±0.159	1.61±0.421	0.839±0.035	1.78±0.375		
15-oxoETE	0.700±0.259	0.657±0.124	0.683±0.114	0.952±0.237	0.470±0.0587	1.050±0.313		
5-iso $PGF_{2\alpha}$VI	–[b]	0.104±0.00738[a]	–[b]	0.0891±0.0381[ab]	0.0682±0.0682[ab]	0.131±0.0138[a]	0.0011	
Sum	184±47.3	271±36.3	205±37.4	237±44.1	151±22.7	249±56.3		0.0446
LA derived oxylipins								
9,10-EpOME	0.0979±0.0273	0.101±0.0276	0.227±0.0528	0.22±0.0742	0.181±0.0735	0.123±0.0671		
12,13-EpOME	0.351±0.064[B]	0.348±0.0677	0.614±0.0888[A]	0.527±0.0731	0.456±0.0966[AB]	0.446±0.168	0.0489	
9,10-DiHOME	1.63±0.300[B]	1.58±0.257	5.51±0.543[A]	5.76±0.275	1.77±0.243[B]	1.70±0.182	<.0001	
12,13-DiHOME	1.24±0.227[B]	1.61±0.392	2.57±0.220[A]	2.35±0.0977	1.55±0.341[B]	1.47±0.218	0.0012	
9-HODE	10.9±1.48	16.3±1.44	18.1±2.60	22.7±3.53	14.2±4.55	18.7±4.73		0.0288
13-HODE	6.67±0.948	11.0±1.33	12.0±1.65	14.8±1.95	7.22±0.959	14.4±3.53		
9-oxoODE	0.368±0.0947[B]	0.287±0.0566	0.945±0.127[A]	0.879±0.196	0.586±0.208[A]	0.932±0.323	0.0006	
13-oxoODE	2.77±0.379[B]	2.19±0.337	6.74±0.748[A]	6.18±1.20	3.67±0.723[A]	5.79±1.65	0.0001	
9,12,13-TriHOME	3.02±0.518[B]	4.42±0.744	5.51±1.19[A]	7.44±1.46	5.45±1.69[A]	8.67±2.61	0.0303	0.0323
Sum	27.0±3.41[B]	37.7±4.11	52.2±6.16[A]	60.8±8	33.9±6.85[B]	52.2±13.2	0.0045	0.0273
GLA derived oxylipins								
13-HOTrE-γ	0.293±0.0694	0.304±0.0767	0.437±0.106	0.509±0.0917	0.320±0.106	0.365±0.0700		

DGLA derived oxylipins							
PGD_1	0.174±0.0617	0.344±0.083	0.149±0.0205	0.294±0.0667	0.125±0.0354	0.227±0.0656	0.0301
$PGF_{1\alpha}$	0.0494±0.0227	0.102±0.0324	0.0238±0.0238	0.0779±0.0314	0.0373±0.0184	0.0551±0.025	
8-HETrE	0.039±0.00401	0.0938±0.017	0.0774±0.0102	0.0957±0.0173	0.0697±0.0169	0.0964±0.0166	0.0031
15-HETrE	0.375±0.0588	1.04±0.167	0.447±0.0879	0.63±0.15	0.375±0.0713	0.669±0.132	0.0004
Sum	0.637±0.128	1.58±0.273	0.697±0.134	1.10±0.246	0.607±0.109	1.05±0.221	0.0008
Sum n-6	212±47.3	311±39.4	259±43.1	299±50.1	186±28.4	302±65.8	0.0346
n6/n3 Ratio	13.5±2.34^{B}	14.4±0.916	18.1±1.16^{A}	20.1±2.63	8.95±0.616^{C}	12.2±1.07	<.0001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
[#] Denotes that oxylipin is not detected.
Values are mean±SE (n=5-6 for each), and are based on ng/ml serum.

Table 3.8b. Serum N-3 Oxylipins In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
				ng/ml				
ALA derived oxylipins								
9-HOTrE	0.527±0.0947^{B}	0.833±0.129	0.596±0.110^{B}	0.732±0.130	1.42±0.576^{A}	1.50±0.400	0.0084	
13-HOTrE	0.946±0.205^{AB}	1.27±0.215	0.829±0.169^{B}	0.954±0.147	1.82±0.563^{A}	2.29±0.689	0.0095	
9-oxoOTrE	0.121±0.0144^{B}	0.0974±0.0205	0.131±0.0195^{B}	0.144±0.035	0.204±0.0612^{A}	0.279±0.087	0.0228	
12,13-DiHODE	0.123±0.0261	0.105±0.0261	0.14±0.0207	0.0888±0.00709	0.163±0.0225	0.117±0.0221		0.0435
Sum	1.72±0.321^{B}	2.30±0.38	1.70±0.271^{B}	1.92±0.294	3.61±1.21^{A}	4.18±1.16	0.0097	
EPA derived oxylipins								
$PGF_{3\alpha}$	0.212±0.0616^{AB}	0.0406±0.0105	0.274±0.0719^{A}	0.0896±0.039	0.130±0.0339^{B}	0.0278±0.00783	0.0088	<.0001
TXB_3	0.0429±0.00922	0.104±0.0218	0.0337±0.00524	0.0459±0.0069	0.0384±0.0118	0.086±0.0179		0.0001
5-HEPE	0.0575±0.00951	0.141±0.0207	0.0660±0.0101	0.095±0.0124	0.0902±0.0138	0.146±0.023		0.0002
8-HEPE	0.0301±0.00457^{A}	0.0402±0.00574	0.028±0.00643^{B}	0.0211±0.00315	0.0472±0.0107^{A}	0.0505±0.00676	0.0022	
12-HEPE	1.93±0.4^{AB}	5.04±1.20	2.19±0.576^{B}	2.12±0.376	3.62±0.673^{A}	5.36±1.09	0.0266	0.022
15-HEPE	0.0605±0.00748^{A}	0.155±0.0253	0.0399±0.00631^{B}	0.0679±0.00861	0.0785±0.0116^{A}	0.182±0.0399	<.0001	<.0001
18-HEPE	0.0614±0.00984^{A}	0.118±0.0241	0.0476±0.0125^{B}	0.0663±0.00952	0.0991±0.0252^{A}	0.188±0.0454	0.0027	0.0039
Sum	2.4±0.458^{AB}	5.64±1.25	2.68±0.602^{B}	2.51±0.383	4.1±0.74^{A}	6.04±1.19	0.0282	0.025
DHA derived oxylipins								
4-HDoHE	1.02±0.155	1.07±0.150	0.747±0.157	0.763±0.133	1.05±0.157	1.12±0.201		
7-HDoHE	0.203±0.0158	0.156±0.0238	0.153±0.0266	0.143±0.0201	0.205±0.0305	0.177±0.037		
8-HDoHE	0.253±0.0346	0.243±0.0518	0.168±0.0306	0.162±0.0261	0.247±0.0396	0.254±0.0389		
10-HDoHE	0.246±0.0272	0.247±0.025	0.206±0.0397	0.176±0.0308	0.221±0.0323	0.243±0.0612		
11-HDoHE	0.104±0.013	0.131±0.021	0.0742±0.0192	0.11±0.0196	0.101±0.0197	0.146±0.0382		
13-HDoHE	0.456±0.0367	0.511±0.0519	0.337±0.0604	0.371±0.0679	0.426±0.0616	0.511±0.105		
14-HDoHE	4.05±0.675	5.10±0.86	3.61±0.698	3.44±0.676	3.68±0.548	4.70±1.40		
16-HDoHE	0.284±0.0232	0.303±0.0361	0.23±0.0448	0.249±0.043	0.316±0.0458	0.302±0.0587		
17-HDoHE	3.32±0.199^{AB}	4.93±0.716	3.14±0.373^{B}	4.03±0.539	5.25±0.626^{A}	5.54±1.21	0.0424	
20-HDoHE	0.488±0.0602	0.573±0.117	0.498±0.121	0.602±0.118	0.662±0.0954	0.697±0.188		
19,20-DiHDoPE	0.520±0.0689	0.468±0.0726	0.349±0.0664	0.390±0.0585	0.442±0.0363	0.637±0.103		
16,17-EpDPE	0.0883±0.0214	0.152±0.0234	0.0572±0.0364	0.113±0.0506	0.085±0.042	0.112±0.0598		
19,20-EpDPE	0.0849±0.0103	0.0849±0.00948	0.0845±0.0181	0.0774±0.00661	0.0884±0.0182	0.0731±0.0106		
Sum	11.1±0.689	14±1.68	9.66±1.24	10.6±1.54	12.8±0.773	14.5±3.18		
Sum n-3	15.2±1.01^{AB}	21.9±2.9	14±1.87^{B}	15.1±2	20.5±2.34^{A}	24.7±5.25	0.0181	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
Values are mean±SE (n=5-6 for each), and are based on ng/ml serum..

Table 3.9. Serum Total Fatty Acids In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

Diet	Adequate LA		High LA		High LA+ALA			
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
				ug/ml				
C140	26.8±1.94^{A}	32.5±7.19	25±6.84^{A}	39.2±7.11	10.9±1.91^{B}	19.9±4.66	0.009	
C160	826±107^{A}	753±59.3	419±92.7^{B}	561±87.2	677±70.4^{AB}	586±94.5	0.0073	
C161	78.3±12.5^{ba}	103±17.4^{a}	16.8±3.28^{d}	54.9±8.65^{bc}	34.8±4.64^{c}	67.7±13.9^{ba}	0.045❖	
C170	5.37±1.15	3.22±0.940	4.23±1.35	1.81±0.120	4.08±0.97	3.27±0.630		
C180	640±77.1^{A}	345±39.7	377±51.7^{B}	313±61.2	665±44.3^{A}	305±59.9	0.0335	<.0001
C181	1293±192^{A}	976±146	295±68.6^{C}	365±66.5	606±58.9^{B}	477±65.7	<.0001	
C182n6	548±54.6^{B}	645±45.5	750±158^{AB}	842±106	1036±101^{A}	839±135	0.0178	
C183n3	27.1±3.2^{B}	38.7±5.12	23.1±5.78^{B}	25.3±4.11	68.7±10.7^{A}	62.8±12.0	0.0001	
C183n6	8.42±1.24	7.57±1.01	7.26±0.630	5.69±1.05	9.52±0.73	5.46±0.950		0.0108
C200	4.85±0.33	5.86±0.510	4.27±0.860	4.26±0.58	5.37±0.49	4.94±0.760		
C201	7.58±1.27^{A}	9.37±1.57	1.69±0.640^{C}	3.72±0.900	4.23±0.46^{B}	4.61±1.08	<.0001	
C202n6	4.09±0.260^{c}	7.54±0.440^{bc}	9.14±2.39^{b}	13.8±1.29^{a}	8.94±0.470^{b}	10.2±1.49^{ab}	0.0031	
C203n3	0.48±0.1^{B}	0.87±0.29	2.35±1.91^{A}	5.49±2.14	0.81±0.1^{AB}	2.65±1.11	0.0371	0.0125
C203n6	16±1.44^{b}	27.4±4.65^{a}	10.7±1.52^{c}	14.5±1.98^{bc}	20.02±0.97^{ba}	16.5±3.21^{bc}	0.0413	
C204n6	884±117	605±68.3	591±60.7	602±105	885±91.7	594±107		0.0208
C205n3	11.0±1.55^{A}	10.5±1.81	2.82±0.460^{B}	1.93±0.23	13.6±0.85^{A}	6.83±1.03	<.0001	0.0063
C220	6.41±0.32^{B}	7.48±0.38	6.33±0.87^{B}	8.06±0.82	10.7±0.55^{A}	10.3±1.77	0.0006	
C221	0.910±0.06	0.770±0.200	0.640±0.120	0.330±0.270	0.870±0.0800	0.540±0.110		
C222n6	0.700±0.660	0.480±0.380	0.110±0.0800	0.160±0.160	0.770±0.660	0.120±0.0800		
C224n6	6.96±0.410^{B}	9.91±0.59	23.2±4.32^{A}	23.3±4.08	7.82±0.63^{B}	12.8±1.86	<.0001	0.0155
C225n3	5.21±0.66^{B}	10.2±1.01	5.55±0.93^{B}	7.43±1.02	9.3±0.62^{A}	12.6±1.79	0.0017	0.0009
C225n6	20.5±2.17^{A}	18.1±2.78	13.9±1.41^{A}	23.8±3.43	11.9±1.15^{B}	11.2±3.07	0.0064	
C226n3	152±18.0^{A}	79.7±5.78	84.5±11.8^{B}	59.4±10.1	173±16.8^{A}	68.4±9.55	0.0018	<.0001
C240	12.1±1.73	10.7±0.600	11.3±1.35	10.8±1.57	13.4±0.43	10.6±1.95		
C241	12.7±0.94	13.8±1.81	8.45±0.73	11.1±1.57	10.4±1.31	12.8±4.06		
n6/n3 Ratio	7.35±0.515^{B}	9.50±0.581	11.9±0.461^{A}	14.9±0.811	7.48±0.221^{B}	8.94±0.717	<.0001	0.0001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
Values are mean±SE (n=5-6 for each), and based on ng per ml of serum.

3.3.2 High LA Compared To Adequate LA Effects On Oxylipins

Increasing the level of dietary LA from 2 to 5% resulted in higher n-6 fatty acid derived oxylipins (Table 3.4a, 3.6a, 3.8a), even when the precursor level was not increased (Table 3.5, 3.7, 3.9). This was most clearly exhibited in the kidney, where EDA was the only n-6 that was higher in the high LA rats, yet oxylipins derived from LA (4), AA (12), and 1 each from GLA, DGLA, EDA and AdA were higher (Table 3. 4a). Other n-6 derived oxylipins that were not significantly elevated followed the same trend, as evidenced by the higher levels of total LA (83% higher), total AA (35% higher) and total n-6 derived oxylipins (63% higher) in the high compared to adequate LA groups. In liver, high LA increased the levels of LA and AA, but not GLA, DGLA or AdA. This was accompanied by higher levels of oxylipins from LA (7), AA (11), AdA (2) and 1 each from GLA and EDA, as well as higher levels of total LA (175% higher) and total n-6 fatty acid derived oxylipins (82% higher) (Table 3. 6a). Serum exhibited only elevated levels of EDA and AdA in high compared to adequate LA feeding, but exhibited higher levels of oxylipins derived from LA (6) and AA (3), as well as higher levels of total LA oxylipins (75% higher) (Table 3. 8a).

A higher level of dietary LA had fewer effects on n-3 fatty acid derived oxylipins, although the effect varied by tissue. In kidney, high compared to adequate LA reduced EPA and DHA levels, but not ALA (Table 3. 5). Yet, 1 ALA derived oxylipin (12,13-DiHODE) and 19,20-DiHDPE derived from DHA were lower in kidneys of high LA fed rats (Table 3. 4b). In the liver, only EPA was lower (Table 3. 7), but 2 ALA oxylipins were higher in the high LA group, and 1 EPA and 1 DHA oxylipin was lower (Table 3. 6b). In the serum, EPA and DHA, but not ALA, were lower in the high LA group (Table 3. 9), but only 3 EPA oxylipins were lower (Table 3. 8b).

A higher level of LA also had effects on non-enzymatically produced AA oxylipins. In kidney 2 out of 3 (5-iso $PGF_{2\alpha}$VI and 8-iso 15keto $PGF_{2\beta}$) were ~28%-38% higher in

the high LA group (Table 3. 4a,). Similar to kidney, in liver 2 out of 2 non-enzymatically produced AA oxylipins (5-iso $PGF_{2\alpha}$VI and 8-iso $PGF_{2\alpha}$III) were higher (~70% -80%) with high LA intake (Table 3. 6a). In serum there was 1 non-enzymatic AA oxylipin detected (5-iso $PGF_{2\alpha}$VI) but there was no significant difference in its level between the adequate LA and high LA groups (Table 3. 8a.).

3.3.3 High LA+ALA Compared To Adequate LA Effects On Oxylipins

When both LA and ALA were increased in the diet to maintain a similar LA/ALA ratio, the effect of elevating n-6 oxylipins followed the same pattern as high dietary LA alone, although fewer n-6 fatty acid derived oxylipins were elevated. In the kidney, similar to the high LA diet, AA levels were not different in the high LA+ALA compared to adequate LA diet, but the high LA+ALA diet resulted in higher levels of oxylipins derived from LA (6), AA (5) and 1 each from GLA and DGLA, as well as higher levels of total LA (121% higher), total AA (23% higher) and total n-6 fatty acid derived oxylipins (79% higher) (Table 3. 4a). In liver, high LA+ALA increased LA fatty acid level but not AA, and it also increased n-6 fatty acid oxylipins derived from LA (7), AA (4), EDA (1), as well as resulting in higher levels of total LA (150% higher) and total n-6 fatty acid derived oxylipins (59% higher) (Table 3. 6a). Similar to the fewer effects of high LA on n-6 fatty acid derived oxylipins in serum compared to kidney and liver, the effects of high LA+ALA in serum on these oxylipins also were fewer: only 4 LA and 1 AA derived oxylipin in serum were higher in the high LA+ALA compared to the adequate LA diet (Table 3. 8a).

The high LA+ALA diet had a much greater effect on n-3 fatty acid derived oxylipins than the high LA diet, when both were compared to the adequate LA diet, particularly in the kidney. In the kidney, the high LA+ALA diet increased 4 ALA, 5 EPA and 7 DHA oxylipins, as well as total ALA (185% higher), total EPA (51% higher), total DHA (38%

higher) and total n-3 fatty acid derived oxylipins (47% higher) when compared to the adequate LA group (Table 3. 4b.). These higher levels of EPA and DHA oxylipins were present even though there were no differences in EPA or DHA levels between the LA+ALA and adequate LA groups. In the liver and serum, the LA+ALA diet also affected n-3 fatty acid derived oxylipins, but the differences were fewer than in the kidney. In the liver, only oxylipins derived from ALA (3) as well as total ALA derived oxylipins were higher (278% higher) (Table 3. 6b). Again, these were higher despite a lack of any differences in the levels of ALA or EPA between these two groups. In serum, ALA was increased by the LA+ALA diet, and so were 3 ALA as well as total ALA oxylipins (202% higher), while neither EPA and DHA fatty acid, nor oxylipin levels, were different in the LA+ALA compared to the adequate LA diet (Table 3. 8b).

A higher level of LA+ALA also had effects on non-enzymatically produced oxylipins. In kidney 2 out of 3 (5-iso $PGF_{2\alpha}$VI and 8-iso 15k $PGF_{2\beta}$ from AA) were ~30% higher in the high LA+ALA group (Table 3. 4a). However in liver or serum there were no significant differences on the non-enzymatically derived oxylipins.

3.3.4 Sex Effects

Sex differences were observed in 40-50% (30/75 oxylipins in kidney, 29/74 in liver and 37/73 in serum) of oxylipins (Table 3. 4a, 4b, 6a, 6b, 8a, 8b). Out of these, almost all of these were higher in male, with the following exceptions: in kidney, 20-HETE, $PGF_{3\alpha}$, Δ^{17}-6-keto $PGF_{1\alpha}$, and all DHA derived oxylipins were higher in females; in liver, tetranor-12-HETE, 12-oxo-ETE, $PGF_{1\alpha}$ and 13-HDoHE were higher in females; in serum, 20-HETE, 12,13-DiHODE, and $PGF_{3\alpha}$ were higher in females. The higher oxylipin levels in males did not coincide with fatty acid levels, however. In the kidney, all 3 n-3 fatty acid were higher in females, while the only n-6 fatty acid that displayed a sex effect was DGLA, which was higher in males (Table 3. 3.4b, 3.5). In liver, AA and DHA were

higher in females, while LA, GLA and DGLA were higher in males (Table 3.6a, 3.6b, 3.7). In serum, GLA, AA, EPA and DHA were higher in females, while AdA was higher in males (Table 3.8a, 3.9).

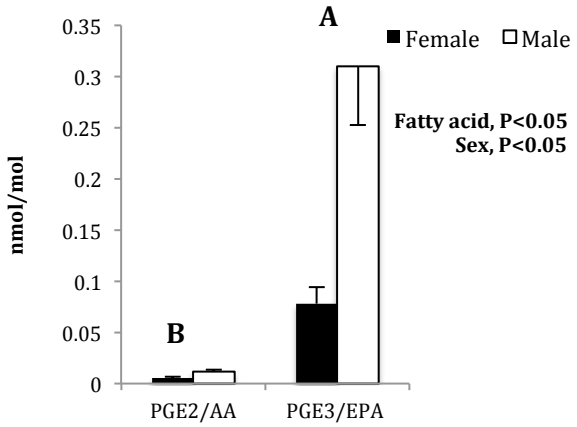
To account for the differing fatty acid levels on oxylipin formation, the ratios of oxylipins to parent phospholipid fatty acid were determined for oxylipins formed from multiple fatty acids via the following pathways: COX/PGE synthase, 5-, 12-, and 15-LOX, CYP hydroxylase (fatty acid hydroxylated at n-2 selected), and CYP epoxygenase (Figure 3. 5. a-f, Table 3.10. a-f). These analyses revealed that oxylipin formation in males was greater than or similar to females for all oxylipins for all fatty acid substrates. This was also the case for DHA derived oxylipins in kidneys, which were higher in females despite having greater than or similar oxylipin/DHA levels for males.

3.3.5 Effect Of Fatty Acid Type And Chain Length On Oxylipin Formation

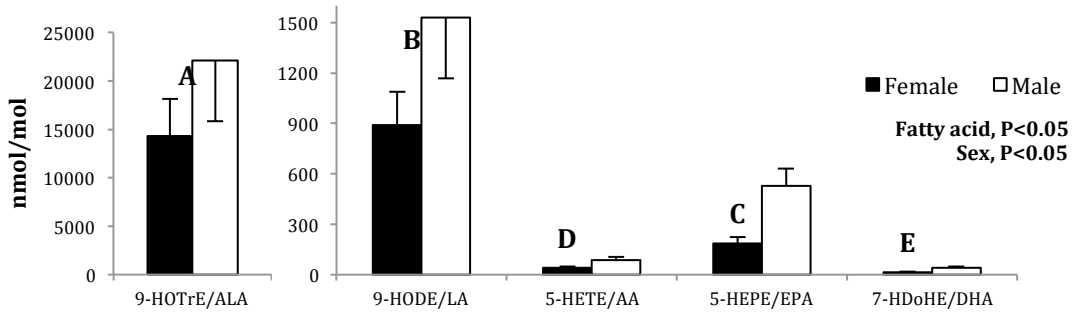
The product to fatty acid ratios (Tables 3.10. a-f) also were used to examine the relative rates of oxylipin formation from different fatty acids. The pattern was similar in all pathways, with oxylipin formation being higher from n-3 compared to n-6 fatty acids. In addition, the order of fatty acid conversion to oxylipins was 18-carbon > 20-carbon ≥ 22-carbon fatty acid, with the exception of the CYP hydroxylase pathway, where 20-HDoHE/DHA was higher than 18-HEPE/EPA. An example (liver high LA+ALA group) is presented in graphical format in Figure 3. 5. a-f.

Figure 3.5. Product/Precursor Ratios In Rat Livers Given High LA+ALA Diets For Six Weeks.

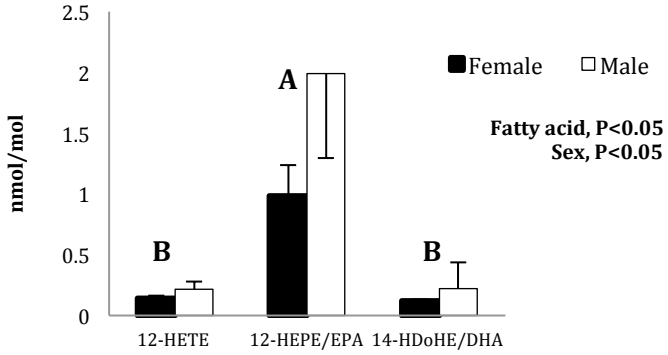
5a. COX-PGE synthase



5b. 5-LOX

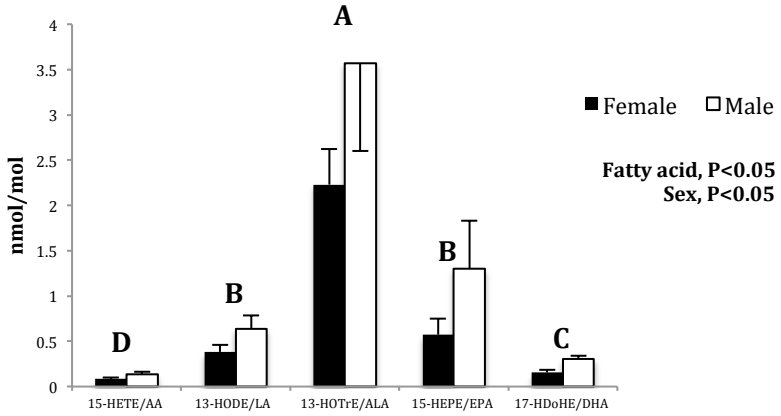


5c. 12-LOX

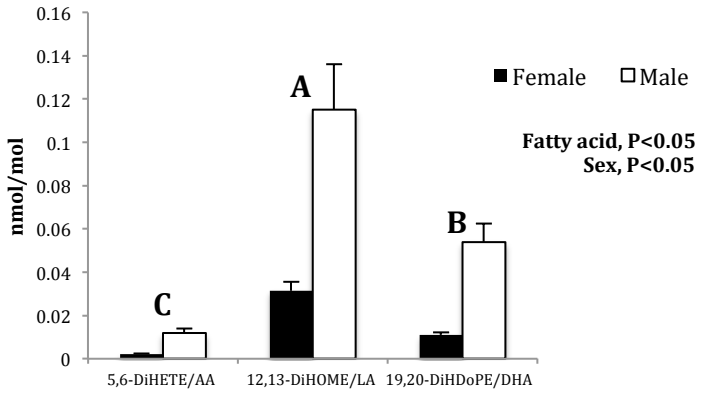


Differing upper case superscript letters shown on the female values indicated differences between diets.
Differing lower case superscript letters differences between means.
n=5-6 for each.

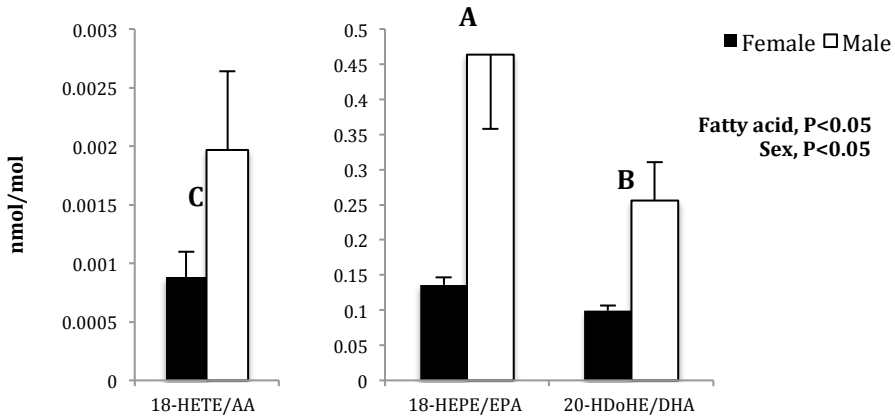
5d. 15-LOX



5e. CYP Epoxygenase



5f. CYP Hydroxylase



Differing upper case superscript letters shown on the female values indicated differences between diets.
Differing lower case superscript letters differences between means.
n=5-6 for each.

Table 3.10. Product/Precursor Ratios For Enzymes In Rats Given Adequate LA, High LA And High LA+ALA Diets For Six Weeks.

a. COX-PGE synthase

		PGE_2/AA		PGE_1/DGLA		PGE_3/EPA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Adequate LA	8.37±1.46^{C}	8.12±1.41	24.3±5.35^{B}	27.0±4.11	90.3±18.7^{A}	135±28	<.0001	
	High LA	9.75±1.04^{C}	8.38±1.27	38.9±2.88^{B}	41.7±5.98	178±46.3^{A}	267±33	<.0001	
	High LA+ALA	8.80±2.61^{C}	7.86±1.13	33.7±9.03^{B}	39.5±6.53	200±101^{A}	195±23.9	<.0001	
Liver	Adequate LA	7.01±1.53^{c}	14.1±1.54^{c}			49.1±17.7^{b}	272±44^{a}	0.0271❖	
	High LA	8.46±1.72^{B}	10.1±0.700			286±88^{A}	441±123	<.0001	
	High LA+ALA	5.67±0.85^{B}	11.7±1.95			78.1±16.0^{A}	310±57.3	<.0001	<.0001
Serum	Adequate LA	3.85±1.09	10.5±3.04						0.0491
	High LA	7.00±2.66	13.5±5.38						
	High LA+ALA	5.03±2.81	8.17±1.71						

Differing upper case superscript letters shown on the female values with in a row indicated differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=4-6)

b. 5-LOX

		5-HETE/AA		9-HODE/LA		9-HOTrE/ALA		5-HEPE/EPA		7-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol										Fatty acid	Sex
Kidney	Adequate LA	111±12.5^{ef}	161±17.4^{e}	1010±96.2^{c}	1070±168^{c}	3200±749^{b}	8910±2020^{a}	649±132^{d}	1400±105^{c}	68.6±9.52^{g}	86.5±6.68^{fg}	0.0086❖	
	High LA	49.2±0.32^{C}	77.7±0.55	400±164^{B}	617±176	954±3.58^{A}	3400±4.11	706±6.92^{B}	844±1.8	33.1±0.44^{D}	173±2.17	<.0001	0.0438
	High LA+ALA	160±22.7^{C}	182±21.5	1610±334^{B}	2110±376	3480±688^{A}	9290±2590	874±115^{B}	2030±508	129±19.6^{C}	113±6.30	<.0001	0.0016
Liver	Adequate LA	1.07±0.44^{f}	2.07±0.47^{e}	751±164^{b}	980±176^{b}	7860±2250^{a}	10400±2400^{a}	188±45.9^{c}	568±195^{b}	19.8±4.01^{d}	31.4±3.32^{d}	<.0001	0.0009
	High LA	62.2±16.7^{D}	83.4±7.29	1440±381^{B}	1330±173	4720±1220^{A}	10300±2000	612±178^{C}	872±200	31.6±11^{E}	42.5±11.6	<.0001	0.0275
	High LA+ALA	41.1±6.47^{D}	86.6±19.3	891±196^{B}	1530±362	14300±3870^{A}	22100±6250	187±37.9^{C}	530±101	16.2±1.14^{E}	40.4±8.85	<.0001	<.0001
Serum	Adequate LA	2.41±0.32^{d}	5.55±0.55^{c}	25.6±6.92^{a}	24.1±1.8^{a}	18.8±3.58^{ba}	21.9±4.11^{a}	4.97±0.44^{c}	13.7±2.17^{b}	1.33±0.14^{e}	1.87±0.26^{ed}	0.0065	
	High LA	4.36±1.00^{B}	8.85±1.94	31.4±9.03^{A}	29.6±7.65	37.2±11.9^{A}	33.2±13.9	29.6±10.4^{A}	51.1±6.84	1.98±0.54^{C}	2.37±0.48	<.0001	
	High LA+ALA	2.1±0.08^{B}	8.6±2.06	9.6±1.85^{A}	16.2±3.76	14.7±4.99^{A}	17.7±5.92	6±1.22^{A}	22.4±3.85	1.79±0.77^{C}	2.62±0.52	<.0001	<.0001

Differing upper case superscript letters shown on the female values with in a row indicated differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

Values are mean±SE (n=4-6)

c. 12-LOX

		12-HETE/AA		12-HEPE/EPA		14-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Adequate LA	41.2±2.25^{B}	61±6.67	222±26.3^{A}	420±38.7	282±31.7^{A}	386±56.4	<.0001	0.0001
	High LA	78.3±16.8^{B}	97.8±11.1	512±132^{A}	947±222	372±37.7^{A}	607±104	<.0001	0.0069
	High LA+ALA	98±25.1^{B}	106±20.0	464±95^{A}	901±108	780±198^{A}	662±100	<.0001	
Liver	Adequate LA	168±36.5^{B}	187±44.3	927±85.2^{A}	920±209	148±35.6^{B}	184±32.5	<.0001	
	High LA	92±43.3^{B}	138±20.6	2540±958^{A}	2020±451	161±66.3^{B}	142±22.9	<.0001	
	High LA+ALA	140±23.1^{B}	217±63.5	986±253^{A}	1990±697	121±13.2^{B}	219±41.3	<.0001	0.025
Serum	Adequate LA	140±34.9^{A}	279±41.9	194±47.6^{A}	523±153	27.9±6.63^{B}	63±12.9	<.0001	0.0002
	High LA	247±63.4^{B}	305±60.1	909±339^{A}	1190±191	47.2±13^{C}	63.2±10.4	<.0001	
	High LA+ALA	102±15.3^{B}	289±41.9	217±35.7^{A}	761±132	18.5±2.63^{C}	63.8±14.8	<.0001	<.0001

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Values are mean±SE (n=4-6)

d.15-LOX

		15-HETE/AA		13-HODE/LA		15-HETrE/DGLA		13-HOTrE/ALA		15-HEPE/EPA		17-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male		
Tissue	**Diet**	**nmol/mol**												**Fatty Acid**	**Sex**
Kidney	**Adequate LA**	114±10.7^{g}	122±30.7^{g}	557±85.1^{dc}	681±113^{c}	374±13.2^{de}	0.303±0.0297^{fe}	1530±257^{b}	3760±960^{a}	219±20.2^{f}	376±41.5^{de}	453±71.3^{dce}	490±86.1^{dce}	0.0331❖	
	High LA	209±12.8^{D}	227±43.3	1230±89.5^{C}	1300±185	579±30.7^{B}	558±104	2150±336^{A}	4480±957	516±41.6^{C}	698±75.7	652±77.9^{C}	1120±348	<.0001	
	High LA+ALA	157±29.4^{D}	167±32.4	992±192^{B}	1440±298	508±94.2^{C}	611±133	2420±424^{A}	6090±1610	358±16.5^{C}	693±98.8	1360±422^{B}	825±167	<.0001	0.0221
Liver	**Adequate LA**	79.1±19.1^{D}	189±38.3	332±85.2^{C}	463±91.6			1050±170^{A}	1810±412	549±28.9^{B}	544±104	104±16.6^{D}	200±25.6	<.0001	0.002
	High LA	88.5±13.8^{f}	101±8.43^{fe}	725±198^{d}	548±80.5^{d}			12300±3830^{a}	1860±235^{b}	1220±429^{cd}	1580±444^{cb}	189±54.1^{fe}	191±34.3^{e}	0.0011	
	High LA+ALA	85.2±12.4^{D}	136±25.4	380±78.9^{B}	636±147			2230±393^{A}	3570±970	572±178^{B}	1300±529	154±29.4^{C}	304±35.2	<.0001	0.0012
Serum	**Adequate LA**	9.11±2.24^{d}	23.4±3.74^{bc}	15.6±4.02^{c}	16.2±1.66^{c}	23.5±4.53^{bc}	42.6±12^{ba}	35.2±10.0^{b}	33.8±7.26^{b}	5.69±0.82^{d}	14.9±1.81^{c}	22.4±3.02^{bc}	58.9±7.05^{a}	0.0253	
	High LA	14.8±4.28^{e}	27.7±7.87^{bdec}	20.7±5.79^{de}	18.8±4.45^{dec}	49.6±15.7^{bac}	58.2±12.3^{ba}	49.7±15.9^{bac}	42.1±16.2^{bdac}	16.4±4.9^{e}	0.0407±0.00714^{bac}	37.6±4.96^{bac}	71.9±10.9^{a}	0.0003	0.0102
	High LA+ALA	11.9±5.91^{B}	23±3.32	7.04±1.32^{B}	23.1±10.8	12±4.07^{B}	42.4±7.77	14.1±7.62^{B}	24.1±6.17	9.3±4.3^{B}	27.1±5.49	37.6±6.17^{A}	80.9±14.8	<.0001	<.0001

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Values are mean±SE (n=4-6)

e. CYP Hydroxylase

		18-HETE/AA		18-HEPE/EPA		20-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Adequate LA	0.52±0.10^{B}	0.41±0.08	603±104^{A}	787±127	760±102^{A}	990±110	<.0001	
	High LA	0.52±0.12^{B}	0.59±0.05	1440±119^{A}	1000±187	934±140^{A}	1130±188	<.0001	
	High LA+ALA	0.53±0.07^{c}	0.49±0.07^{c}	869±116^{b}	1600±260^{a}	1640±316^{a}	1180±116^{ab}	0.0246❖	
Liver	Adequate LA	1.01±0.23^{B}	2.95±0.74	161±35.4^{A}	408±149	104±28.4^{A}	251±47.9	<.0001	0.0003
	High LA	2.95±1.19^{C}	2.34±0.87	636±115^{A}	508±117	144±51.7^{B}	215±32.1	<.0001	
	High LA+ALA	0.88±0.220^{C}	1.97±0.67	136±11.1^{A}	464±106	99.1±7.25^{B}	256±54.8	<.0001	<.0001
Serum	Adequate LA	0.16±0.02^{C}	0.29±0.04	5.42±0.56^{A}	11±1.89	3.13±0.29^{B}	6.79±1.31	<.0001	<.0001
	High LA	0.36±0.10^{C}	0.53±0.08	21.9±10.0^{A}	33.5±7.80	6.57±2.24^{B}	8.81±1.42	<.0001	0.0145
	High LA+ALA	0.180±0.03^{C}	0.82±0.25	5.39±0.84^{A}	28.9±6.67	3.26±0.35^{B}	10.1±2.36	<.0001	<.0001

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Values are mean±SE (n=4-6)

f. CYP Epoxygenase

		5,6-DiHETE/AA		12,13-DiHOME/LA		12,13-DiHODE/ALA		19,20-DiHDoPE/DHA		Fatty acid	Sex
		Female	Male	Female	Male	Female	Male	Female	Male		
Tissue	Diet	nmol/mol								Fatty Acid	Sex
Kidney	Adequate LA	1800±320^{C}	2280±500	10800±2230^{B}	8870±1780	65500±16000^{A}	167000±50900	18800±6670^{B}	14200±2640	<.0001	
	High LA	2710±1060^{C}	3330±760	10200±900^{B}	12500±1580	59900±14100^{A}	61200±12800	12100±820^{B}	14500±1790	<.0001	
	High LA+ALA	2240±660^{D}	3020±820	10200±1440^{C}	14900±3850	74900±7700^{A}	179000±53900	24200±5890^{B}	15000±1440	<.0001	
Liver	Adequate LA	3740±1400^{C}	12200±2790	32000±8080^{A}	121000±23800			12200±2750^{B}	68800±11200	<.0001	<.0001
	High LA	3090±410^{C}	14500±3280	43600±9870^{A}	157000±42500			11400±1850^{B}	53400±9670	<.0001	<.0001
	High LA+ALA	2240±290^{C}	12000±1980	31400±4220^{A}	115000±21000			10900±1160^{B}	54000±8450	<.0001	<.0001
Serum	Adequate LA	430±80^{e}	1780±370^{d}	2440±410^{bcd}	2260±580^{cd}	4240±1220^{a}	3090±570^{cd}	3290±560^{bc}	5680±1290^{a}	0.0006❖	
	High LA	950±210^{c}	2540±550^{b}	3860±800^{ab}	2530±580^{b}	6960±2170^{a}	4500±960^{ab}	4390±1300^{ab}	5940±890^{a}	0.0407	
	High LA+ALA	200±100^{d}	2680±1010^{c}	3360±930^{bc}	2120±730^{c}	58800±22400^{a}	1740±500^{c}	1170±380^{c}	9430±1710^{b}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicated differences between diets. Differing lower case superscript letters within a row indicate differences between means.

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Values are mean±SE (n=4-6)

3.4 Discussion

3.4.1 Effect Of High LA And High LA+ALA Intake

The results of the present study demonstrate that rat oxylipin levels are not necessarily correlated to tissue phospholipid or serum total fatty acid levels. A high LA intake, which is compared to adequate LA intake, resulted in a pronounced increase in LA and AA oxylipins as well as other n-6 derived oxylipins in kidney, liver and serum, while the phospholipid LA and AA levels were only higher in liver. High LA intake did not increase LA or AA levels in kidney or serum.

Previous findings in rodents and humans showed that dietary LA affects LA oxylipin production (18, 34, 35). For example lowering LA from 6.7%E to 2.4%E for 12 weeks significantly reduced the abundance of LA oxylipins (9- and 13- HODEs and oxo-ODEs) in human plasma (18). Similarly in rats, plasma LA oxylipins were increased by 3-8 fold when provided at 5.2%E and 10.5%E compared to 0.4%E (34). In rat kidney of obese rats on a high fat diet, LA oxylipins were 6-7 fold higher when rats were provided diet with 73% compared to 8% LA (35). Consistent with this research, in our study most LA oxylipins in kidney, liver and serum were significantly increased by ~80%-~400% in the high LA (~11.5%E) compared to adequate LA (~4.6%E) group, even though LA only increased in liver but not in kidney or serum.

There is controversy on whether high amounts of LA intake belong in a healthy diet. LA in adipose tissue has increased by 136% over the last half century which reflects an increased LA intake (2). One point of view is that increasing LA will increase eicosanoids, which are metabolites from AA, and contribute to the pro-inflammatory effect of n-6 fatty acids. But there is almost no evidence to support this because the conversion from LA to AA is tightly regulated by delta-6 desaturase so that the AA level

does not change with high LA intake. However our data shows that besides increasing LA oxylipins, an increased LA intake is also related to higher levels of AA derived oxylipins.

Many AA derived oxylipins were increased after a high LA intake without necessarily affecting AA phospholipid levels. For example some prostanoid metabolites, HETEs and their metabolites and leukotrienes were increased after high LA intake, but AA was only higher in liver. So, this discrepancy between changes of fatty acid and changes of oxylipins indicates that the fatty acid level does not always reflect oxylipin levels, and the fatty acid composition alone provides an incomplete representation of the oxylipin profile.

AA is of particular interest because it plays many important roles in cell signaling and is considered a pro-inflammatory fatty acid. It has been suggested that LA is not converted to AA derived oxylipins because changes in dietary LA do not affect plasma AA levels. This is supported by a systematic review that showed that when dietary LA intake varies from 90% lower to 550% higher, plasma AA levels does not change (36). This is consistent with the current result in kidney and serum that show that when LA intake was increased from 4.6%E to 11.5%E, the level of AA did not change, but many AA oxylipins in these tissues were increased. A few very recent studies, have examined the levels of AA metabolites in response to dietary LA. In rat brain, extreme lowering of dietary LA intake to 0.04%E from 5%E reduced the LPS-induced increase in PGE_2 and COX activity (37), and PGE_2 and 11,12,15-TriHETrE in rat peripheral tissues (38). A very high level of LA in high fat diets also increased DiHETrE and HETE in rat kidneys of obese rats (35). The current study demonstrates that in kidney, liver and serum of normal rats, a moderate increase in dietary LA from an adequate (4.6%E) to a higher level (11.5%E) increased the levels of many AA oxylipins, even when AA levels were not increased.

The reason why higher LA can increase AA oxylipins without changing AA levels

is still unclear. One reason may be a higher LA intake level induces an increased flux from LA to AA and to AA oxylipins without changing the AA concentration. A diet-induced increase in AA also may be offset by increased β-oxidation or increased metabolic conversion of AA into bioactive mediators (39, 40), keeping AA levels constant.

Besides increasing a large number of n-6 oxylipins, high LA also affects n-3 fatty acid derived oxylipins. A high LA intake decreased ALA, EPA and DHA oxylipins (e.g. 12,13-DiHODE, 19,20-DiHDoPE in kidney, PGE_3 and 4-HDoHE in liver, 8-, 15- and 18-HEPE in serum) in kidney, liver and serum, but the number of changes is much less than for n-6 oxylipins. EPA levels in kidney, liver and serum were lower in the high LA group; DHA levels were lower in kidney and serum, but not liver. This result is consistent with previous research that shows that an increase of dietary LA from 0.4%E to 5%E and 10%E can decrease EpDPE and EpETrE (epoxy-eicosateteaenoic acid) in rat peripheral tissues (38), as well as in another study that showed that EPA and DHA oxylipins were lower with 5%E and 10%E LA feeding compared to 0.4%E (34). In our study, increasing the LA content in diet from 4.6%E to 11.5%E reduced the EPA and DHA levels in tissues. The reduced precursor fatty acid may have induced a lower EPA and DHA oxylipin level.

High dietary LA with adequate ALA increased many n-6 fatty acid derived oxylipins and decreased some n-3 derived oxylipins. Similarly, high LA+ALA compared to adequate LA diets did not increase AA levels, but LA in kidney, liver and serum was higher in the high LA+ALA group. The high LA+ALA compared to adequate LA diet still had similar effects on oxylipin production; however, the changes were not as many as with the high LA diet. There were 26 n-6 fatty acid derived oxylipins in kidney, 25 in liver and 11 in serum that were higher in the high LA group compared to the adequate LA group, but there were 12 in kidney, 14 in liver and 5 in serum that were higher in the high LA+ALA compared to the adequate LA group. Increasing ALA from 0.5%E to

1.4%E resulted in ~50% fewer changes, suggesting that a small amount of ALA can mitigate the effect of high LA on n-6 fatty acid derived oxylipins. To date there is no research on the effect of combining high LA with different levels of ALA on oxylipins. Our research shows that adding ALA to a high LA diet can reduce the elevated levels of n-6 fatty acid derived oxylipins in tissues, but at the level included in the current diets, did not abolish all differences. Adding ALA to a high LA diet does not reduce tissue LA levels, so the competition does not appear to be at the fatty acid level. The competition may be between fatty acid conversions to oxylipins. The product/substrate ratios provide evidence that conversion of ALA to its oxylipin products is favored over the production of LA oxylipins from LA.

Unlike the effect of high compared to adequate LA diets on reducing some n-3 fatty acid derived oxylipins, high LA+ALA increased many n-3 derived oxylipins when compared to both the adequate LA and the high LA group. This is consistent with flax oil feeding studies that have demonstrated increased EPA and DHA oxylipins (27, 35). Interestingly, high LA+ALA compared to both adequate and high LA diets resulted in elevated EPA and DHA oxylipin levels, even though EPA and DHA fatty acid levels were similar in high LA+ALA and adequate LA groups. The ratio of LA/ALA was similar in the adequate LA and in the LA+ALA diets, indicating that the amount of ALA and LA in the diet affects long chain n-3 fatty acid oxylipin production, while the fatty acid levels appear to be more closely related to the LA/ALA ratio. This is consistent with our previous study in obese rats provided isocaloric high fat diets with differing LA/ALA ratios (35). So, dietary ALA can increase EPA and DHA derived oxylipins. Many n-3 fatty acid derived oxylipins have beneficial effects, which are reviewed by Gabbs et al (41). For example, 18-HEPE from EPA can inhibit macrophage-mediated inflammation (42); 14-HDoHE from DHA can inhibit platelet aggregation (43) and so forth. Several studies have shown that adding ALA to the diet could have similar effects on n-3 oxylipins as adding DHA (27, 35). These data have shown that dietary ALA can increase

the levels of EPA and DHA oxylipins and thus may mediate similar health effects as dietary fish oil containing EPA and DHA; however, the relative efficacy of dietary ALA compared to dietary EPA and DHA in elevating EPA and DHA oxylipins remains to be examined.

From the clinical trials that have been done on oxylipins, the most commonly used samples are human plasma, serum or urine. However it is unclear whether the alterations observed are the same in all tissues. Our study indicates that kidney and liver compared to serum are more responsive to dietary n-6 fatty acids. Similar results were seen in previous rat studies (34, 44) that showed the alterations observed in different tissues are not consistent. Our research therefore suggests that the serum oxylipin profile may not reflect the profile in tissues. The reason for the tissue-specific differences in oxylipin levels is unclear. It may be due to different compartmental half-lives or incorporation from plasma into tissues, or differences in metabolism in different locations resulting in uptake, degradation, turnover and oxidation rates that are different. This has implications for clinical trials, because blood is the most commonly used sample and is used to draw conclusions related to clinical outcomes. One of the very few human studies that focused on dietary LA and oxylipin production showed that blood eicosanoid levels are unchanged with LA intake (45), which is consistent with our result that dietary LA intake has fewer effects on rat serum. It may not, however, necessarily reflect tissue oxylipins.

To summarize this section, our study showed that either high LA or high LA+ALA can increase n-6 oxylipins and decrease n-3 oxylipins without necessarily altering fatty acid levels. Adding a small amount of ALA to the high LA diet can mitigate the high LA effect on lowering n-6 fatty acid oxylipins and enhance n-3 fatty acid oxylipins. Finally, oxylipin production is tissue-specific.

3.4.2 Sex Effect And Tissue Specificity

The discrepancy of fatty acid and oxylipin levels indicates that fatty acid level does not always reflect the oxylipin profile. This same discrepancy was observed in the effects of sex. For example, kidney DHA and DHA oxylipins are both higher in females, while AA levels are higher but AA oxylipins are lower in females.

To date there are few studies that have examined sex effects on oxylipin production. Spontaneously hypertensive female rats have higher PGE_2 and TXA_2 levels than males in urine, and testosterone decreases PGE_2 production in renal inner medulla (28). A sex effect on oxylipins was observed in female mice that displayed higher levels of LTC_4 and LTB_4 and lower LT biosynthesis with androgen treatment in peritoneal macrophages (29). Rat renal eicosanoids were lower in male compared to female normal rats (46). Also in humans, the level of 11d-TXB_2 is higher in females and PGEM is higher in urine in males (47). The reasons for these sex differences in oxylipins may be due to differences on enzymes, oxylipin receptors or enzyme locations. A sex-related phospholipase activity has been shown to be suppressed by testosterone, resulting in different leukotriene synthesis in human monocytes (48). Other oxylipin related enzymes, such as 9-ketoreductase, CYP4A, CYP2C23, CYP epoxygenase and 5-LOX also are affected by sex (46, 49-51). Among those, 9-ketoreductase and CYP4A, CYP2C23 are higher in females and CYP epoxygenase is higher in males. Also receptors may be altered by sex. For example, TXA_2 receptors are increased by treatment with testosterone(52). Furthermore, enzyme location may be affected by sex, as shown with 5-LOX differences in stimulated whole blood or neutrophils in humans. Testosterone appears to alter 5-LOX subcellular localization and cause decreased leukotriene synthesis (53).

The differences in sex effects on oxylipins also may be due to fatty acid substrate levels. For example, renal DHA is higher in females and DHA oxylipin levels also are higher in females, even though the DHA oxylipin to DHA ratios in males are higher than

or similar to females, indicating that substrate level may be important to produce oxylipins. On the other hand, males have higher oxylipin levels than females and almost all of the enzymes with a sex effect have higher product/substrate levels in males. Thus even though the liver AA level is higher in females, almost all AA derived oxylipins are higher in males. This indicates that the rate of conversion of fatty acid to oxylipin more than compensates for the lower AA level in many cases in males. To conclude, both conversion rate and fatty acid precursor level contribute to the differences in oxylipin levels in males and females.

In the liver, one exception to the pattern of higher oxylipin levels in males was observed in the metabolites of 12-HETE (tetranor 12-HETE, 12-oxoETE), which were higher in females, suggesting that the metabolism of 12-HETE may be higher in females. In addition, 20-HETE in kidney and serum, and its metabolite (20 COOH AA in liver) were often higher in females than males. Clearly further studies are needed to understand the mechanisms by which sex alters oxylipin levels.

In addition to sex specific production of oxylipins, their production also displays tissue specificity. Comparison of the different tissues revealed that the pattern of n-6 fatty acid derived oxylipin changes in response to dietary LA in the kidney and liver were similar to each other, but dissimilar to serum. The high LA diets resulted in only ¼ to ½ as many higher n-6 fatty acid derived oxylipins in serum as compared to kidney and liver. A similar discrepancy was observed when comparing the effects of dietary ALA on n-3 fatty acid derived oxylipins, but in this case, the changes were much greater in the kidney, compared to liver and serum.

3.4.3 Fatty Acid Incorporation And Enzyme Selectivity

Selectivity of oxylipin levels occurs not only with sex, but also with different fatty acids (Table 3. 10 a-f, Figure 3. 5. a-f). When the product/substrate ratios for enzymes in several oxylipin biosynthetic pathways were examined, these ratios indicated a higher selectivity for n-3 fatty acid over n-6 fatty acid conversion to their respective metabolites. Similar findings were shown in obese rats (35), and in studies of CYP selectivity of product formation from EPA and DHA compared to AA (44, 54, 55). These findings and the fact that a higher proportion of ALA is metabolized via β-oxidation (56), may partly explain why ALA levels are lower than LA in tissues. Additionally, these ratios show that these enzymes convert C18 fatty acids more readily than their longer chain fatty acid counterparts, consistent with previous findings (57, 58).

Interestingly, the LA/ALA ratio is much higher in tissues than in the diet, suggesting that dietary LA is incorporated into tissue phospholipid more effectively, or is metabolized via β-oxidation less readily than ALA. The current study also suggests that ALA is converted to oxylipins preferentially, possibly also contributing to the lower ALA compared to LA in tissues.

In conclusion, high dietary LA compared to adequate LA resulted in higher levels of many LA and AA oxylipins and lower levels of some n-3 oxylipins; high dietary LA+ALA also resulted in higher levels of n-6 oxylipins, but mitigated some of the effects of high LA. The fatty acid level did not consistently reflect oxylipin levels. There are many sex effects on oxylipins, with males generally having higher levels of oxylipins than females. The conversion of fatty acids to oxylipins is higher in males compared to females, in n-3 compared to n-6 fatty acids and in shorter chain compared to longer chain PUFA. Therefore, oxylipin metabolism does not necessarily reflect fatty acid composition, so conclusions regarding the formation of these fatty acid metabolites in some contexts may need to be re-considered. For example, dietary advice to increase LA

intake may need to be re-considered in light of its effect on the production of pro-inflammatory bioactive lipids from both LA and AA.

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Chapter 4 Effect of dietary n-3 fatty acids on rat kidney, liver and serum oxylipin profiles

4.1 Introduction

There is considerable debate on whether α-linolenic acid (ALA) can provide all the benefits of longer-chain n-3 fatty acids (FA). The key question is whether ALA can be sufficiently converted to docosahexaenoic acid (DHA) for optimal health, since fatty acid data show that the conversion from ALA to DHA is poor. In non-human primates ALA conversion to DHA is reported be 0.23% and 0.57% (1, 2), and in humans the conversion of ALA to DHA varies from 0.05%-4% (3, 4), based on fatty acid composition. DHA has beneficial health effects and is critical for tissues such as the retina and brain (5, 6). The benefits of DHA are related to the bioactive lipids produced from this fatty acid, such as resolvins, protectins and hydroxy DHA (HDoHE) that possess anti-inflammatory, vasodilatory, anti-aggregatory and anti-proliferative effects (7-10). Our laboratory has shown that dietary ALA can be converted to EPA and DHA oxylipins in a model of renal disease in which DHA and DHA oxylipins are reduced (11). In addition to conversion, retroconversion also occurrs at the fatty acid level from DHA to EPA (12-15). However there is no evidence for the retroconversion from EPA to ALA or DHA to ALA. Research showed that feeding DHA did not increase ALA level (16), and feeding DHA even decreased ALA in pig liver and muscle (16) and in human serum (17). With EPA feeding, ALA level did not change in pig liver and muscle and decreased in human serum (16, 17). These studies indicate that there is no retroconversion from EPA to ALA or DHA to ALA. Whether ALA is converted to DHA oxylipins and whether EPA or DHA feeding will affect ALA oxylipins in normal kidneys or other tissues remains to be elucidated.

In addition, there are differences between the biological actions of different n-3 fatty acid. For example, fish oils but not ALA decrease inflammation and prevent cardiac dysfunction in the pressure overload-induced rat model (18). Also, fish oils are preferable to ALA in order to increase levels of EPA and DHA in adults with ADHD, and to

decrease the AA/EPA ratio (19). There also are differences in the biological actions of EPA and DHA. DHA is more effective than EPA in decreasing inflammation markers such as C-reactive protein, IL-6 and TNF-α, decreasing triacylglycerides and increasing HDL cholesterol (20), while EPA but not DHA shows an effect on improving depression (21) and increasing mitochondrial fatty acid oxidation (22). DHA compared to EPA also improves postprandial arterial stiffness and the different oxylipin profiles produced from EPA and DHA associated with these vascular effects suggest that these oxylipins may mediate these events (23). It suggests that the different effect of EPA and DHA may be related to their derived oxylipins. However all these studies have used a combination of EPA and DHA (24-26) to examine their effect on oxylipin production, so the effects of individual fatty acids on oxylipins is unclear.

The interconversion of n-3 fatty acid and the production of bioactive lipids (oxylipins) from these fatty acid therefore have implications for dietary intake. Many organizations worldwide provide recommendations for minimal DHA intake (27, 28), while others do not provide a minimum recommendation for DHA, and only have a recommendation for adequate ALA intake (29, 30). Similarly, many recommendations for EPA and DHA do not make specific recommendations for EPA or DHA (27, 31-34). There is much data on how dietary fatty acid can alter tissue fatty acid, but data on how individual n-3 fatty acid affect oxylipins from these fatty acid are lacking. Since these bioactive lipids mediate many of the physiological and health effects of these fatty acid, an understanding of how dietary n-3 fatty acid affect oxylipins will provide further data relevant to dietary recommendations for these fatty acid. Also, there are very few studies that have examined the effect of sex on oxylipin production. It has been shown in a few studies that female rats have higher PGE_2 and TXA_2 levels than males and testosterone decreases PGE_2 production in renal medulla (35). Female mice showed a higher level of LTC_4 and LTB_4 with androgen treatment in peritoneal macrophages (36). However previous research only has examined several individual oxylipins, and there is no study that has

investigated the sex effect on oxylipins derived from all PUFA. The objective of the current study was to compare the effects of dietary ALA, EPA and DHA on the oxylipin profile in kidney, liver and serum in normal rats.

4.2 Materials And Methods

4.2.1. Rats And Diet

Six male and six female healthy weanling Sprague-Dawley rats were provided 4 different diets, for a total of 48 rats. In each diet group, they were provided diets based on the AIN93G diet except that the diets contained 10 g instead of 7 g oil/100g diet, and the diet ingredients, and the oil source varied between diets as outlined below and in Table 4. 1. The fatty acid composition for dietary oils is listed in appendix 1.1; the diet fatty acid composition is calculated based on this. Step by step details for making the diet is listed in appendix 2.1. Protocol for analyzing fatty acid composition in oils is listed in appendix 2.2. This level of fat resulted in diets with 23% of energy as fat. The mixture of oil sources in the 4 diets resulted in similar saturated and unsaturated fatty acid composition in all diets. The control diet had adequate levels of LA and ALA without any EPA or DHA. Note that the control diet group in this chapter is the same rats as the adequate LA diet group in chapter 1. The ALA, EPA and DHA diets each differed from the control diet by having 3g/100g diet more ALA, EPA and DHA, respectively, than the control diet. The higher ALA, EPA and DHA in these diets primarily replaced monounsaturated fatty acid in the control diet.

Rats were weighed weekly and were terminated after 6 weeks of feeding. Rats were anesthetized with isofluorane and terminated via decapitation to collect trunk blood, which was centrifuged at 800 g to obtain serum, and stored at at -80°C until analysis. The right kidney and a portion of the liver were removed, immediately frozen in liquid

nitrogen, and stored at -80°C until analysis. All procedures were performed in accordance with the Canadian Council for Animal Care guidelines and approved by the University of Manitoba Animal Care Committee.

Table 4.1. Diet Composition And Fatty Acid Content Of Diets.

	Control	ALA	EPA	DHA
Diet Ingredient		**g/100g diet**		
Cornstarch	34.9	34.9	34.9	34.9
Casein (87% protein)	20.7	20.7	20.7	20.7
Dextrinized cornstarch	13.7	13.7	13.7	13.7
Sucrose	10.3	10.3	10.3	10.3
Fiber	5.2	5.2	5.2	5.2
Mineral mix (AIN93G)	3.6	3.6	3.6	3.6
Vitamin mix (AIN 93)	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3
Choline bitart	0.3	0.3	0.3	0.3
TBHQ	0.002	0.002	0.002	0.002
DHA oil	-	-	-	3.3
EPA oil	-	-	3.3	-
Oilve oil	7	-	-	-
Soy oil	2.2	1.3	3.8	3.8
Coconut oil	0.65	2.3	2.9	2.9
Flax oil	0.15	6.4	-	0
Total Diet	100	100	100	100
Fatty acid in the diet		**g/100g diet**		
LA	2.13	1.77	2.09	2.09
ALA	0.27	3.43	0.27	0.27
EPA	0	0	3.14	0.01
DHA	0	0	0.01	3.14
SFA	1.93	2.39	2.64	2.65
UFA	6.63	6.75	6.62	6.55
MUFA	4.22	1.53	0.99	0.98
PUFA	2.41	5.22	5.63	5.58
LA/ALA	7.76	0.52	7.63	7.66
n6/n3 Ratio	7.74	0.52	0.64	0.63

Cornstarch, Casein (87% protein), Dextrinized cornstarch, Sucrose, Fiber, Mineral mix (AIN93G), Vitamin mix (AIN 93), L-Cystine, Choline bitart, Oilve oil, Soy oil, Coconut oil, Flax oil were purchased from Dyets, Inc, Bethlehem, PA. EPA fish oil and DHA fish oil were purchased from Larodan, Solna, Sweden. TBHQ was purchased from Sigma-Aldrich, Inc. the diet fatty acid composition is also calculated based on Appendix 1.1.

4.2.2. Oxylipin Analysis

Kidney and livers were lyophilized and a portion was homogenized in Tyrode's salt solution (pH 7.6). Step by step protocol of lyophilisation and homogenization is listed in appendix 2.3 and 2.4. Samples for oxylipin analysis were prepared and analyzed by LC-MS/MS multiple-reaction monitoring as described (37). Oxylipins scanned but not detected are listed in Appendix 1.2.b. Oxylipins without primary are listed in Appendix 1.3, including their mass transition and expected time based on the experimentally detected time difference of deuterated standard and retention times published (38). Dose response curves were run to determine detector response factors, which were applied to all oxylipins, unless otherwise noted when primary standards were unavailable. Oxylipin mass transitions, internal standards, and retention time are listed in Appendix 1.4. HPLC solvent gradient is listed in Appendix 1.5. Deuterated internal standard used for oxylipin analysis is listed in Appendix 1.6. 400 µL of serum was used for oxylipin analysis and 200µL of kidney and liver homogenate was used for oxylipin analysis. Briefly, after adding 10µL of deuterated internal standards (Cayman Chemical, MI, USA) per 400µL serum and per 200µL kidney and liver homogenate, samples were adjusted to pH<3 by using HCl (Sigma-Aldrich, Inc). Solid phase extraction was with Strata-X SPE columns (Phenomenex, CA, USA) that were preconditioned with methanol and pH3 water, loaded with sample, rinsed with 10% methanol, and eluted with methanol. Samples were dried down and resuspended in solvent for analysis by HPLC/MS/MS (Sciex 6500; Sciex, ON, Canada). Quantification of oxylipins was determined using the stable isotope dilution method (39). Dose response curves were run to determine detector response factors, which were applied to all oxylipins, unless otherwise noted when primary standards were unavailable (Appendix 5.1.3). Protocols for solid phase extraction and analysis of oxylipin data from HPLC/MS/MS are listed in appendix 2.5 and 2.6. The amount of oxylipins was expressed as ng of oxylipin per mg dry tissue in kidney and liver, and ng of

oxylipin per mL of serum.

4.2.3. Fatty Acid Analysis

Aliquots of the kidney and liver homogenates (250μL) as described above were used for fatty acid analysis. For homogenates used for fatty acids 8.34μL antioxidant cocktail was added immediately after homogenization. After adding 20μL of internal standard [10μL of C150 (10 mg/mL) of phospholipid, 10μL of free fatty acid C170 (2mg/mL) and triglyceride C170 (5.5mg/mL) in a 1:1 mixture] per 250μL of tissue homogenate or serum, lipids were extracted via solvent-solvent (containing 0.01%BHT) extraction as described (40). Lipid extracts were purified by thin layer chromatography (TLC) (heptane/isopropyl/acetic acid, 60/40/3, v/v/v) to isolate the phospholipid fraction for kidney and liver. After that, fatty acid (phospholipid fatty acid for tissues and total fatty acid for serum) were methylated using methanolic HCl at 80°C for 1 hour and quantified by gas chromatography as described (40). Step by step protocol of fatty acid analysis is listed in appendix 2.7. Fatty acids were expressed as μg per g dry tissue for kidney and liver and μg per mL in serum.

4.2.4. Statistical Analysis

Data were analyzed using SAS 9.3 (SAS Institute Inc, Cary, NC). The Shapiro-Wilk test was used to test for normality. Data were analyzed by using two-way analysis of variance (ANOVA) to test the main effects (diet, sex) or were analyzed using the Kruskal-Wallis test when data could not be normalized by logarithmic transformation. Outliers were removed if the data could not be normalized and the data point was outside of the mean±3SD. The protected LS Means test was used to detect differences between multiple means. All tests were set at a significance level of $P<0.05$. Data are shown as means±SE.

4.3 Results

4.3.1 General Results

All rats grew well, with males having higher body weights throughout the study (Table 4. 2). Body weights were similar throughout the study in rats given the different diets, with the exception of lower body weights of male rats given the DHA diet. There were no differences in feed intake between rats given the different diets (data not shown).

Overall, of the 164 oxylipins scanned for, 83 were detected in kidney, 74 in liver and 72 in serum; those not detected in any tissue are listed in appendix 1.2.b. The proportions of the number of oxylipins derived from different fatty acid was similar in kidney, liver and serum, with approximately two-thirds of oxylipins being derived from n-6 fatty acid, and AA derived oxylipins being approximately two-thirds of all n-6 fatty acid derived oxylipins. Almost half of the n-3 fatty acid derived oxylipins were from DHA, 30-40% were from EPA and ~15% were from ALA (Table 4.4, Table 4.6, Table 4.8).

On a mass basis, however, the distribution of oxylipins differed markedly between tissues, but did not necessarily reflect the fatty acid composition of these tissues. For example, in the control fed rats (Figure 4. 1), oxylipins derived from AA made up 29-36% of oxylipin mass in kidney, 43-44% in liver and 81-83% in serum, while in these same tissues, AA made up 66-67% of fatty acid mass in kidney, 43-44% in liver, and 42-53% in serum. Proportions of fatty acid and their oxylipins for all groups in male and female rats given the control diets are shown in Table 4.3. These proportions changed markedly with the inclusion of ALA, EPA and DHA in the diet, and provide more examples of the discrepancies between levels of tissue fatty acid and oxylipins.

Heat maps of the relative levels of oxylipin profiles in all 3 tissues are shown in Figure 4. 2-4. The raw data for these heat maps is shown in tables 4.4, 4.6, 4.8. Fatty acid

data are in table 4.5, 4.7, 4.9.

Table 4.2. Body Weights In Rats Given Dietary ALA, EPA And DHA Compared To Control For Six Weeks.

	Control		ALA		EPA		DHA			
	Female	Male	Female	Male	Female	Male	Female	Male		
Week	grams								Diet	Sex
0	118±3.0	128±5.1	115±3.2	122±2.5	115±2.4	129±4.9	119±3.2	126±4.3		0.001
1	174±4.6	205±5.0	169±4.7	196±3.5	163±4.1	203±4.9	170±3.3	191±4.6		<.0001
2	215±6.5	279±4.7	208±6.6	265±6.5	199±6.3	282±6.4	212±5.0	261±6.7		<.0001
3	246±8.0^{cd}	349±4.6^{a}	240±8.3^{cd}	336±8.2^{ab}	229±7.1^{d}	344±5.3^{ab}	249±7.7^{c}	320±9.4^{b}		0.0491
4	280±11^{cd}	415±5.0^{a}	268±11^{d}	403±11^{ab}	262±10^{d}	414±7.0^{a}	303±8.9^{c}	379±9.9^{b}		0.002
5	306±12	465±5.5	290±14	452±11	286±13	465±7.6	312±14	423±11		<.0001
6	318±11^{cd}	510±7.3^{ab}	302±15^{d}	495±17^{ab}	306±14^{d}	515±9.5^{a}	341±16^{c}	463±13^{b}		0.0202
Gain*	200±9.2^{c}	382±5.6^{a}	187±14^{c}	374±17^{a}	191±13^{c}	385±13^{a}	222±15^{c}	336±13^{b}		0.0114

Values are mean±SE. Values in same row with differing superscript letters are significantly different.
*Gain represents change in body weight over 6 week feeding period.

Figure 4.1. Example Of The Differences In Oxylipin Compared To Fatty Acid Distributions.

Shown is the distribution of oxylipins compared to fatty acids in kidneys phospholipids of rats provided control diet for 6 weeks. For comparisons for all tissues and diets, see Table 4. 3.

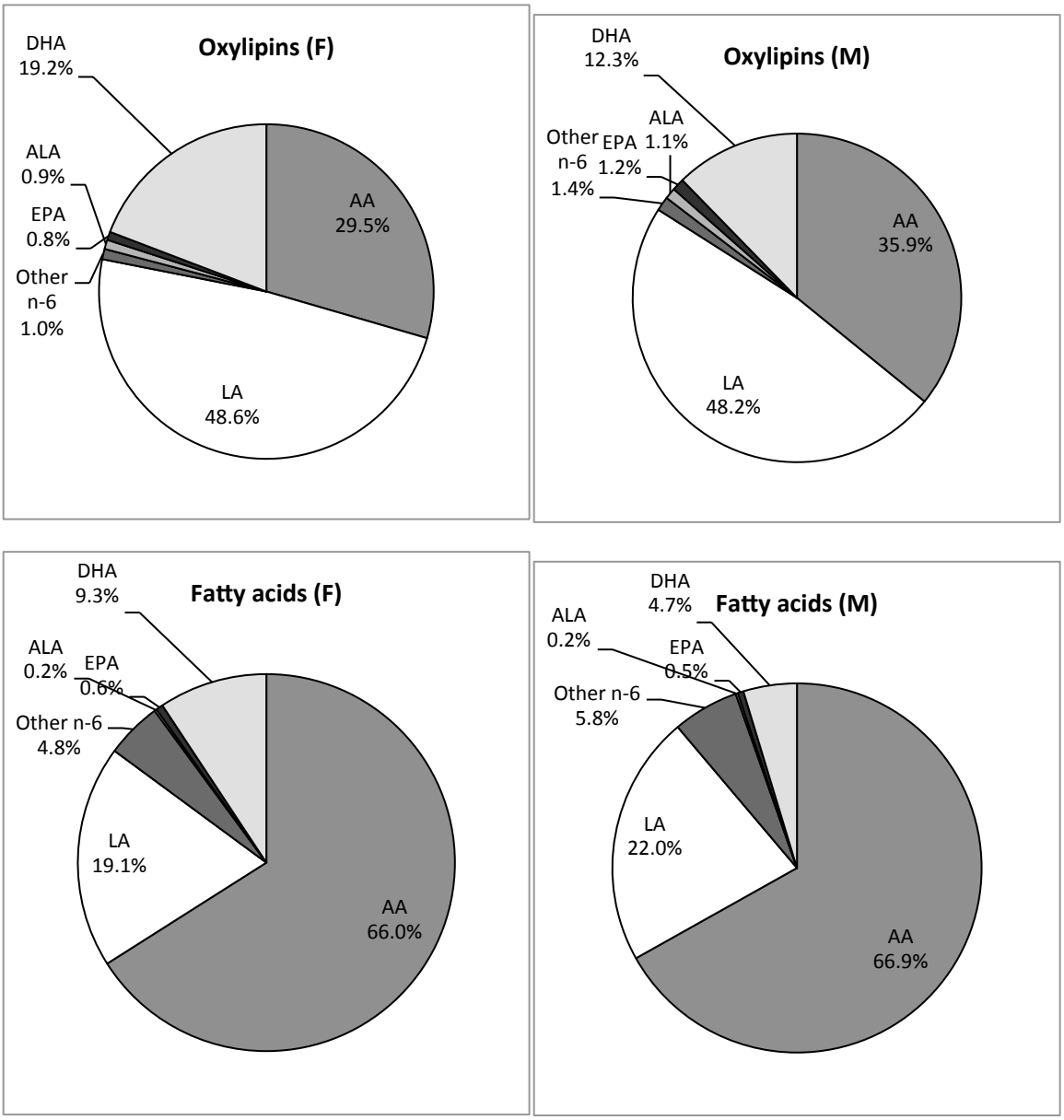


Table 4.3. Differences In Oxylipin Compared To Fatty Acid Mass Distributions For All Tissues And Diets.

Tissue/Group	Sex	Fatty Acid/Oxylipin	AA	LA	Other n-6	ALA	EPA	DHA
					(%)			
Kidney								
Control	**Female**	**Oxylipin**	29.5	48.6	1.0	0.9	0.8	19.2
		Fatty Acid	66.0	19.1	4.8	0.2	0.6	9.3
	Male	**Oxylipin**	35.9	48.2	1.4	1.1	1.2	12.3
		Fatty Acid	67.0	22.0	5.8	0.2	0.5	4.7
ALA	**Female**	**Oxylipin**	15.7	47.2	0.7	9.4	7.4	19.6
		Fatty Acid	44.4	30.3	3.4	3.0	10.7	8.3
	Male	**Oxylipin**	18.9	43.9	0.9	12.2	8.0	16.1
		Fatty Acid	39.3	38.1	4.6	2.6	9.3	6.1
EPA	**Female**	**Oxylipin**	10.8	37.6	0.5	0.7	35.8	14.7
		Fatty Acid	32.0	28.8	2.9	0.4	29.0	6.9
	Male	**Oxylipin**	14.5	38.6	0.5	0.6	35.1	10.7
		Fatty Acid	34.4	31.1	2.9	0.2	26.8	4.5
DHA	**Female**	**Oxylipin**	4.6	24.1	0.3	0.4	6.4	64.3
		Fatty Acid	21.3	34.6	3.2	0.3	19.7	20.9
	Male	**Oxylipin**	4.9	25.9	0.3	0.5	8.3	60.1
		Fatty Acid	20.7	38.8	3.0	0.3	20.6	16.7
Liver								
Control	**Female**	**Oxylipin**	43.3	33.0	2.6	1.5	1.4	18.2
		Fatty Acid	55.7	12.1	7.0	0.1	0.8	24.4
	Male	**Oxylipin**	44.1	32.7	3.7	1.4	1.5	16.6
		Fatty Acid	54.7	16.7	11.1	0.1	0.7	16.7
ALA	**Female**	**Oxylipin**	19.0	24.5	19.0	10.3	15.4	11.9
		Fatty Acid	32.4	20.5	4.8	1.9	18.0	22.5
	Male	**Oxylipin**	16.5	26.9	16.5	5.8	15.6	18.7
		Fatty Acid	30.1	25.2	8.2	1.1	13.2	22.3
EPA	**Female**	**Oxylipin**	9.2	27.1	9.2	1.1	42.8	10.5
		Fatty Acid	26.3	18.9	6.0	0.3	32.0	16.5
	Male	**Oxylipin**	9.3	23.0	9.3	0.8	43.8	13.9
		Fatty Acid	25.2	24.7	7.1	0.3	24.7	18.1
DHA	**Female**	**Oxylipin**	6.4	22.2	6.4	1.1	12.4	51.6
		Fatty Acid	13.3	22.5	5.3	0.2	18.5	40.2
	Male	**Oxylipin**	4.0	18.2	4.0	1.1	10.2	62.5
		Fatty Acid	9.6	28.1	6.3	0.2	12.4	43.5
Serum								
Control	**Female**	**Oxylipin**	81.0	11.9	0.4	0.8	1.1	4.9
		Fatty Acid	52.6	32.7	3.4	1.6	0.7	9.1
	Male	**Oxylipin**	83.3	10.2	0.5	0.6	1.5	3.8
		Fatty Acid	41.7	44.5	4.9	2.7	0.7	5.5
ALA	**Female**	**Oxylipin**	52.7	13.6	0.3	8.1	19.8	5.5
		Fatty Acid	25.7	30.8	2.4	15.3	16.1	9.8
	Male	**Oxylipin**	58.5	10.0	0.5	6.5	17.9	6.6
		Fatty Acid	18.6	39.3	2.8	21.8	9.3	8.2
EPA	**Female**	**Oxylipin**	27.5	7.3	0.2	0.4	62.0	2.7
		Fatty Acid	15.6	30.7	1.4	2.3	44.3	5.7
	Male	**Oxylipin**	29.9	5.7	0.2	0.3	61.1	2.9
		Fatty Acid	14.8	33.0	1.4	2.3	43.3	5.3
DHA	**Female**	**Oxylipin**	27.9	9.4	0.3	0.6	22.1	39.7
		Fatty Acid	9.7	35.9	2.0	2.0	19.1	31.3
	Male	**Oxylipin**	19.0	11.2	0.3	0.8	18.1	50.6
		Fatty Acid	5.0	45.5	1.9	2.7	8.2	36.7

Values are % of total oxylipins and of fatty acid that are precursors to oxylipins

Figure 4.2a. Heat Map Of The Kidney N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

	Diet	Control		ALA		EPA		DHA			
	Sex	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Fatty Acid	Oxylipin	ng/g tissue								Diet	Sex
ALA	9-oxoOTrE									<.0001	
	13-HOTrE									<.0001	
	13-oxoOTrE									<.0001	
	12,13-DiHODE									<.0001	
	Sum									<.0001	
EPA	PGE_3									<.0001	
	Δ^{17} 6k $PGF_{1\alpha}$									<.0001	0.0058
	TXB_3									<.0001	
	5-HEPE									<.0001	0.0225
	8-HEPE									<.0001	
	9-HEPE									<.0001	
	12-HEPE									<.0001	
	15-HEPE									<.0001	
	LXA_5									<.0001	
	18-HEPE									<.0001	
	8-iso $PGF_{3\alpha}$*									<.0001	
	Sum									<.0001	
DHA	17k-DHA									<.0001	
	4-HDoHE									<.0001	
	8-HDoHE									<.0001	0.0006
	10-HDoHE									<.0001	0.0083
	11-HDoHE									<.0001	0.0026
	13-HDoHE									<.0001	0.0008
	14-HDoHE									<.0001	
	16-HDoHE									<.0001	0.0004
	17-HDoHE									<.0001	0.0071
	19,20-DiHDoPE									<.0001	0.0003
	20-HDoHE									<.0001	0.0012
	Sum									<.0001	0.001
	Sum n-3									<.0001	0.0273
	n6/n3									<.0001	0.0088

Legend

Lowest Highest

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

Figure 4.2b. Heat Map Of The Kidney N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

	Diet	Control		ALA		EPA		DHA			
	Sex	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
fatty acid	Oxylipin	ng/g tissue								Diet	Sex
AA	12-HHTrE									<.0001	
	PGA_2									0.0001	
	PGD_2									<.0001	0.045
	$15deoxy\text{-}PGD_2$										
	PGE_2									<.0001	
	$15k\text{-}PGE_2$										
	$6,15\text{-}dk\text{-}dh\text{-}PGF_{1\alpha}$									<.0001	
	$11\beta\text{-}dhk\text{-}PGF_{2\alpha}$									0.0264	
	$PGF_{2\alpha}$									<.0001	
	$6k\text{-}PGF_{1\alpha}$									<.0001	
	$15deoxy\text{-}PGJ_2$									0.0037	0.0349
	TXB_2									<.0001	
	$11d\text{-}TXB_2$									<.0001	
	5-HETE									<.0001	
	5-oxoETE									<.0001	
	5,15-DiHETE									<.0001	
	8-HETE									<.0001	
	9-HETE									<.0001	0.0071
	11-HETE									<.0001	
	12-HETE									<.0001	0.0388
	12-oxoETE									0.0031	
	tetranor-12-HETE									<.0001	
	15-HETE									<.0001	
	8,15-DiHETE									<.0001	
	15-oxoETE									0.0028	0.0355
	HXB_3*									<.0001	
	LTB_4									<.0001	0.0158
	$6t\text{-}LTB_4$									<.0001	
	16-HETE									<.0001	0.0104
	18-HETE										
	20-HETE									0.0022	
	5,6-DiHETrE									<.0001	
	8,9-DiHETrE									<.0001	
	11,12-DiHETrE									<.0001	
	14,15-DiHETrE									<.0001	
	$5\text{-iso } PGF_{2\alpha}VI$									0.0453	
	$8\text{-iso } PGF_{2\alpha}III$									<.0001	
	$8\text{-iso 15k } PGF_{2\beta}$									0.0003	
	Sum									<.0001	
LA	9-HODE										
	9-oxoODE										
	13-HODE									0.0493	
	13-oxoODE										0.0057
	9,12,13-TriHOME										
	9,10-DiHOME										
	12,13-DiHOME										
	Sum									0.0495	
EDA	15-oxoEDE									0.0293	
GLA	13-HOTrE-γ									<.0001	
DGLA	PGE_1									<.0001	
	$PGF_{1\alpha}$									<.0001	
	8-HETrE									0.0083	
	15-HETrE									<.0001	0.0488
	Sum									<.0001	0.0417
AdA	$dihomo\ PGD_2$*									<.0001	
	$dihomo\ PGE_2$*									<.0001	
	$dihomo\ PGF_{2\alpha}$									<.0001	
	Sum n-6									0.0002	

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.
*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

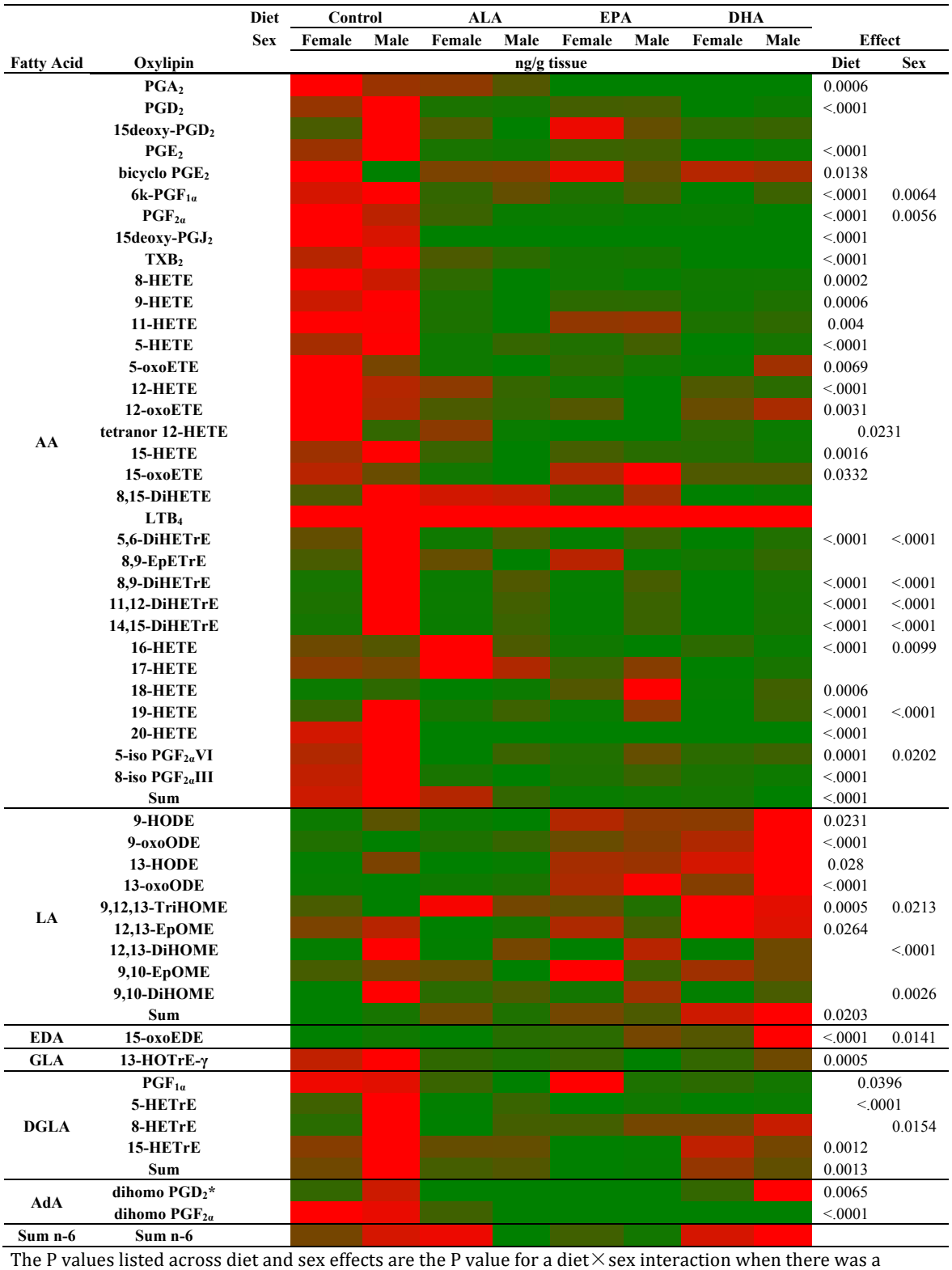
Figure 4.3a. Heat Map Of The Liver N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

		Diet	Control		ALA		EPA		DHA			
		Sex	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Fatty Acid	Oxylipin		ng/g tissue								Diet	Sex
ALA	9-HOTrE										<.0001	
	9-oxoOTrE										<.0001	
	13-HOTrE										<.0001	
	Sum										<.0001	
EPA	PGE_3										<.0001	0.0004
	TXB_3										<.0001	
	5-HEPE										<.0001	0.0017
	8-HEPE										<.0001	
	9-HEPE										<.0001	
	12-HEPE										<.0001	
	15-HEPE										<.0001	
	18-HEPE										<.0001	
	RvE_1										<.0001	
	Sum										<.0001	
DHA	4-HDoHE										<.0001	0.0132
	7-HDoHE										<.0001	
	8-HDoHE										<.0001	
	10-HDoHE										<.0001	
	11-HDoHE										<.0001	
	13-HDoHE										<.0001	
	14-HDoHE										<.0001	
	16-HDoHE										<.0001	
	17-HDoHE										<.0001	
	19,20-DiHDoPE										<.0001	<.0001
	19,20-EpDPE										<.0001	
	20-HDoHE										<.0001	
	Sum										<.0001	
Sum n-3	Sum n-3										<.0001	
	n6/n3 Ratio										<.0001	0.0362

Legend

Lowest Highest

Figure 4.3b. Heat Map Of The Liver N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.



Fatty Acid	Oxylipin	Diet	Control		ALA		EPA		DHA		Effect	
		Sex	Female	Male	Female	Male	Female	Male	Female	Male		
			ng/g tissue								Diet	Sex
AA	PGA_2										0.0006	
	PGD_2										<.0001	
	15deoxy-PGD_2											
	PGE_2										<.0001	
	bicyclo PGE_2										0.0138	
	6k-$PGF_{1\alpha}$										<.0001	0.0064
	$PGF_{2\alpha}$										<.0001	0.0056
	15deoxy-PGJ_2										<.0001	
	TXB_2										<.0001	
	8-HETE										0.0002	
	9-HETE										0.0006	
	11-HETE										0.004	
	5-HETE										<.0001	
	5-oxoETE										0.0069	
	12-HETE										<.0001	
	12-oxoETE										0.0031	
	tetranor 12-HETE										0.0231	
	15-HETE										0.0016	
	15-oxoETE										0.0332	
	8,15-DiHETE											
	LTB_4											
	5,6-DiHETrE										<.0001	<.0001
	8,9-EpETrE											
	8,9-DiHETrE										<.0001	<.0001
	11,12-DiHETrE										<.0001	<.0001
	14,15-DiHETrE										<.0001	<.0001
	16-HETE										<.0001	0.0099
	17-HETE											
	18-HETE										0.0006	
	19-HETE										<.0001	<.0001
	20-HETE										<.0001	
	5-iso $PGF_{2\alpha}$VI										0.0001	0.0202
	8-iso $PGF_{2\alpha}$III										<.0001	
	Sum										<.0001	
LA	9-HODE										0.0231	
	9-oxoODE										<.0001	
	13-HODE										0.028	
	13-oxoODE										<.0001	
	9,12,13-TriHOME										0.0005	0.0213
	12,13-EpOME										0.0264	
	12,13-DiHOME											<.0001
	9,10-EpOME											
	9,10-DiHOME											0.0026
	Sum										0.0203	
EDA	15-oxoEDE										<.0001	0.0141
GLA	13-HOTrE-γ										0.0005	
DGLA	$PGF_{1\alpha}$										0.0396	
	5-HETrE										<.0001	
	8-HETrE											0.0154
	15-HETrE										0.0012	
	Sum										0.0013	
AdA	dihomo PGD_2*										0.0065	
	dihomo $PGF_{2\alpha}$										<.0001	
Sum n-6	Sum n-6											

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

Figure 4.4a. Heat Map Of The Serum N-3 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

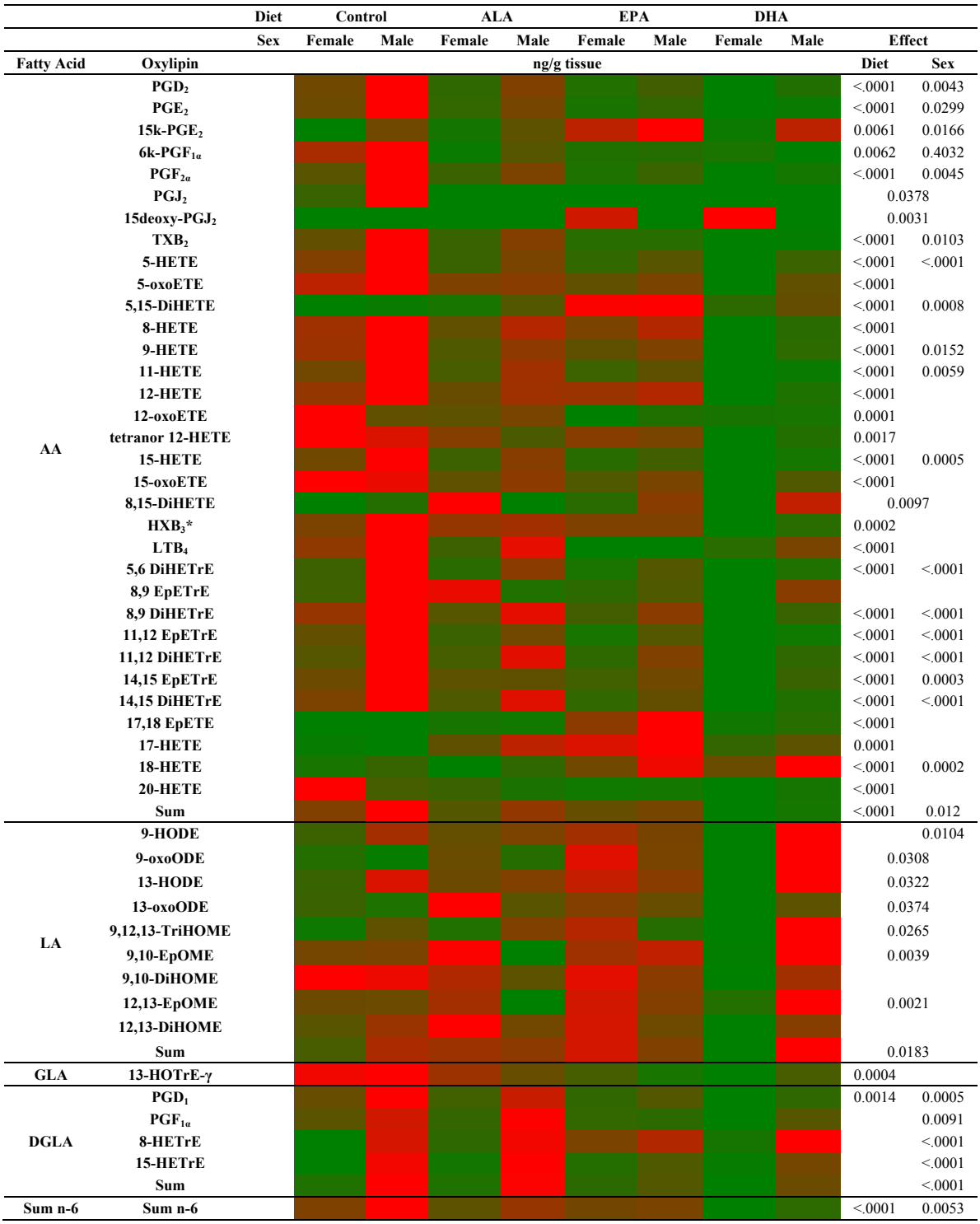
		Diet	Control		ALA		EPA		DHA		Effect	
		Sex	Female	Male	Female	Male	Female	Male	Female	Male		
Fatty Acid	Oxylipin		ng/g tissue								Diet	Sex
ALA	9-HOTrE										<.0001	0.0383
	9-oxoOTrE										<.0001	
	13-HOTrE										<.0001	
	12,13-DiHODE										<.0001	
	Sum										<.0001	
EPA	PGE_3										0.0002	
	$PGF_{3\alpha}$											<.0001
	TXB_3										<.0001	0.0042
	5-HEPE										<.0001	<.0001
	8-HEPE										<.0001	0.0087
	12-HEPE										<.0001	0.0115
	15-HEPE										<.0001	<.0001
	18-HEPE										<.0001	0.0026
	Sum										<.0001	0.0093
DHA	4-HDoHE										<.0001	0.0129
	7-HDoHE										<.0001	
	8-HDoHE										<.0001	
	10-HDoHE										<.0001	0.013
	11-HDoHE										<.0001	0.0042
	13-HDoHE										<.0001	0.0067
	14-HDoHE										<.0001	0.0232
	16-HDoHE										<.0001	0.0192
	17-HDoHE										<.0001	<.0001
	16,17-EpDPE										0.0145	
	19,20-EpDPE										0.0261	
	19,20-DiHDoPE										<.0001	0.0044
	20-HDoHE										<.0001	0.0393
	Sum										<.0001	0.0017
	Sum n-3										<.0001	0.0095
	n6/n3 Ratio										<.0001	

Legend

Lowest Highest

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

Figure 4.4b. Heat Map Of The Serum N-6 Fatty Acid Derived Oxylipin Profile In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.



	Diet	Control		ALA		EPA		DHA			
	Sex	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Fatty Acid	Oxylipin	ng/g tissue								Diet	Sex
AA	PGD_2									<.0001	0.0043
	PGE_2									<.0001	0.0299
	$15k\text{-}PGE_2$									0.0061	0.0166
	$6k\text{-}PGF_{1\alpha}$									0.0062	0.4032
	$PGF_{2\alpha}$									<.0001	0.0045
	PGJ_2									0.0378	
	$15deoxy\text{-}PGJ_2$									0.0031	
	TXB_2									<.0001	0.0103
	5-HETE									<.0001	<.0001
	5-oxoETE									<.0001	
	5,15-DiHETE									<.0001	0.0008
	8-HETE									<.0001	
	9-HETE									<.0001	0.0152
	11-HETE									<.0001	0.0059
	12-HETE									<.0001	
	12-oxoETE									0.0001	
	tetranor 12-HETE									0.0017	
	15-HETE									<.0001	0.0005
	15-oxoETE									<.0001	
	8,15-DiHETE									0.0097	
	HXB_3*									0.0002	
	LTB_4									<.0001	
	5,6 DiHETrE									<.0001	<.0001
	8,9 EpETrE										
	8,9 DiHETrE									<.0001	<.0001
	11,12 EpETrE									<.0001	<.0001
	11,12 DiHETrE									<.0001	<.0001
	14,15 EpETrE									<.0001	0.0003
	14,15 DiHETrE									<.0001	<.0001
	17,18 EpETE									<.0001	
	17-HETE									0.0001	
	18-HETE									<.0001	0.0002
	20-HETE									<.0001	
	Sum									<.0001	0.012
LA	9-HODE										0.0104
	9-oxoODE									0.0308	
	13-HODE									0.0322	
	13-oxoODE									0.0374	
	9,12,13-TriHOME									0.0265	
	9,10-EpOME									0.0039	
	9,10-DiHOME										
	12,13-EpOME									0.0021	
	12,13-DiHOME										
	Sum									0.0183	
GLA	13-HOTrE-γ									0.0004	
DGLA	PGD_1									0.0014	0.0005
	$PGF_{1\alpha}$										0.0091
	8-HETrE										<.0001
	15-HETrE										<.0001
	Sum										<.0001
Sum n-6	Sum n-6									<.0001	0.0053

The P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for Wilcoxin test if the data were not normally distributed.

Table 4.4a. Kidney N-3 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Female	Male	Diet	Sex
					ng/g tissue					
ALA derived oxylipins										
9-HOTrE	347±62.8^{B}	347±42.2	3131±430^{A}	4234±1248	252±44.1^{C}	192±40.7	217±18.4^{BC}	292±75.5	<.0001	
9-oxoOTrE	32.5±3.04^{cd}	70.9±11.2^{b}	316±49.1^{a}	431±198^{a}	39.1±15.6^{cd}	40±10.4^{cd}	24.5±4.48^{d}	56.1±12.9^{cb}	<.0001	
13-HOTrE	156±13.0^{B}	192±26.7	1776±253^{A}	2285±613	174±25.3^{B}	139±39.2	165±14.5^{B}	205±50.7	<.0001	
13-oxoOTrE	–$^{\#B}$	–	2323±451^{A}	2942±1485	–^{B}	–	–^{B}	–	<.0001	
12,13-DiHODE	7.38±1.85^{B}	8.33±2.13	85.1±11.8^{A}	95.9±22.7	7.85±1.96^{B}	3.56±0.972	5.76±1.57^{B}	6.30±1.19	<.0001	
Sum	542±73.4^{B}	618±72.1	7632±948^{A}	9988±3462	473±72.1^{B}	374±89.3	413±34.3^{B}	559±132	<.0001	
EPA derived oxylipins										
PGE_3	23.2±4.28^{C}	24.6±4.01	294±40.1^{B}	293±43.8	1003±73.3^{A}	997±119	287±43.9^{B}	326±21.2	<.0001	
$PGF_{3\alpha}$	20.1±5.37^{d}	6.49±0.814^{e}	52.6±9.27^{cb}	37.3±3.37^{c}	161±15.5^{a}	137±24.6^{a}	61.7±7.74^{b}	57.8±2.47^{b}	0.0202❖	
Δ^{17}-6k-PGF1α	1.88±0.836^{B}	0.87±0.324	11.4±1.16^{B}	8.25±1.43	35.7±4.2^{A}	22.1±3.64	12.3±1.42^{A}	8.56±1.43	<.0001	0.0058
TXB_3	–^{d}	–^{d}	17.9±2.16^{c}	12.8±0.966^{c}	41.7±4.42^{a}	32.9±5.42^{b}	14.4±2.93^{c}	13.8±0.635^{c}	<.0001	
5-HEPE	144±11.8^{C}	251±30	1735±113^{B}	1896±300	8051±1170^{A}	7468±1019	1922±286^{B}	2857±387	<.0001	0.0225
8-HEPE	–^{d}	–^{d}	137±24.9^{dc}	150±36.3^{dc}	629±140^{a}	409±81.7^{b}	155±71.5^{dc}	202±20.6^{c}	<.0001	
9-HEPE	48.1±11.6^{C}	60.1±7.77	577±44.3^{B}	632±121	2519±320^{A}	1893±399	635±177^{B}	908±105	<.0001	
12-HEPE	51.5±5.85^{C}	75.1±9.28	946±144^{B}	1044±181	3091±310^{A}	2778±293	1076±159^{B}	1302±153	<.0001	
15-HEPE	50.8±4.06^{C}	65.6±6.16	591±98.7^{B}	778±117	2543±552^{A}	2136±440	865±135^{B}	1014±162	<.0001	
LXA_5	–^{c}	–^{c}	–^{c}	–^{c}	430±38.1^{a}	426±38.9^{a}	77.4±26.2^{b}	118±14.2^{b}	<.0001	
18-HEPE	138±19.5^{D}	146±30.7	1660±248^{C}	1716±355	6394±1239^{A}	4794±1300	2295±245^{B}	2347±221	<.0001	
8-iso $PGF_{3\alpha}$*	0.00991±0.0027^{d}	0.0244±0.00362^{d}	0.174±0.021^{cb}	0.118±0.0103^{cd}	0.688±0.0428^{a}	0.584±0.082^{a}	0.215±0.0278^{cb}	0.258±0.0397^{b}	<.0001	
Sum	477±15.3^{D}	630±64.9	6023±610^{C}	6567±965	24898±3276^{A}	21095±3403	7401±1001^{B}	9153±621	<.0001	
DHA derived oxylipins										
17k DHA	329±37.6^{BC}	270±43.7	451±96.9^{B}	269±22.4	230±28.1^{C}	213±28.9	1128±193^{A}	1574±317	<.0001	
4-HDoHE	2065±236^{C}	1834±219	3067±575^{B}	2917±515	2230±442^{C}	1651±310	15260±2557^{A}	14085±2118	<.0001	

7-HDoHE	274±28.4^{C}	155±7.88	390±64.8^{B}	332±45.7	253±23.9^{C}	160±25	2324±529^{A}	2040±326	<.0001	0.003
8-HDoHE	465±31.3^{C}	270±31.3	666±86.3^{B}	552±66.3	439±37.2^{C}	270±29.5	3526±612^{A}	3117±484	<.0001	0.0006
10-HDoHE	396±65.5^{C}	181±16.0	449±89.7^{B}	419±75.6	321±53.3^{C}	191±39.2	2741±618^{A}	2516±337	<.0001	0.0083
11-HDoHE	514±52.2^{C}	324±25.6	779±85.9^{B}	623±66.1	529±69.4^{C}	327±43.4	3917±642^{A}	3516±501	<.0001	0.0026
13-HDoHE	836±142^{C}	394±50.9	1069±188^{B}	864±139	680±94.4^{C}	396±58.6	4948±735^{A}	4017±772	<.0001	0.0008
14-HDoHE	1150±129^{C}	692±107	1569±250^{B}	1652±311	1011±145^{C}	636±96.0	7341±1330^{A}	7272±1503	<.0001	
16-HDoHE	563±85.8^{C}	275±27.8	798±142^{B}	605±75.7	536±75.7^{C}	296±49.6	4038±504^{A}	3315±518	<.0001	0.0004
17-HDoHE	1834±308^{C}	781±189	2715±467^{B}	2050±445	1959±328^{BC}	1287±278	15129±2114^{A}	13433±1988	<.0001	0.0071
19,20-DiHDoPE	72±18.2^{B}	26.6±4.17	54.4±6.63^{B}	37.4±6.39	32.8±4.77^{C}	21.8±4.37	194±15.4^{A}	156±31.1	<.0001	0.0003
20-HDoHE	3019±257^{C}	1798±185	4380±631^{B}	3148±192	2194±306^{D}	1383±207	15256±2628^{A}	12974±2330	<.0001	0.0012
Sum	11194±1021^{C}	6734±686	15947±2322^{B}	13230±1308	11459±746^{C}	7222±948	74809±10902^{A}	66568±9011	<.0001	0.001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

# Denotes that oxylipin is not detected.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 4.4b. Kidney N-6 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
					ng/g tissue					
AA derived oxylipins										
12-HHTrE	1490±248^{A}	1440±195	955±154^{B}	1010±276	324±12.3^{C}	436±72.4	560±235^{C}	256±26	<.0001	
PGA_2	140±34.7^{A}	142±11.8	122±26.6^{A}	129±22.1	111±49.8^{B}	56.2±23.2	231±36.9^{A}	215±40.2	0.0001	
PGD_2	235±33.1^{A}	299±56.9	189±33.9^{AB}	244±22.0	170±26.0^{B}	198±35.9	55.7±8.50^{C}	69.1±3.91	<.0001	0.045
15deoxy-PGD_2	32.1±4.20^{AB}	45.8±12.5	41.8±9.26^{A}	46.7±3.94	37.4±6.84^{AB}	44.4±7.53	26.8±7.64^{B}	27.0±4.17		
PGE_2	263±40.8^{a}	231±38.9^{ab}	168±30.3^{bcd}	193±25.7^{abc}	108±10.1^{ed}	127±15.5^{dc}	49.2±3.73^{e}	52.8±3.88^{e}	<.0001	
15k-PGE_2	13.5±2.52	28±5.88	20.6±3.33	39.4±13.7	27.7±2.83	36.1±8.13	21.5±4.88	24.9±8.31		
6,15-dk-dh-$PGF_{1\alpha}$	–$^{\#dc}$	–^{d}	–^{d}	–^{d}	0.873±0.218^{ba}	1.1±0.18^{a}	0.465±0.199^{bc}	1.23±0.264^{a}	<.0001	
11β-dhk-$PGF_{2\alpha}$	115±26^{B}	127±17.1	184±16.9^{A}	175±23.8	160±15.5^{A}	187±29.8	148±13.4^{AB}	133±19.7	0.0264	
$PGF_{2\alpha}$	203±21.2^{A}	269±33.4	115±23.4^{B}	128±13.4	68.1±16.8^{C}	88.7±19.9	48.3±8.58^{C}	51.3±5.06	<.0001	
6k-$PGF_{1\alpha}$	184±19.1^{A}	219±35.2	138±21.2^{B}	146±22.1	67.4±8.75^{C}	72.1±11	44±5.48^{D}	30.3±1.56	<.0001	
15deoxy-PGJ_2	40.2±4.87^{A}	55.3±6.92	33.9±6.59^{A}	51.3±7.89	41.1±5.39^{A}	52.3±6.45	25.2±8.51^{B}	23.4±5.85	0.0037	0.0349
TXB_2	168±36.4^{A}	145±17.7	98.7±16.4^{B}	88.0±7.83	34.9±2.85^{C}	38.2±6.49	23.9±3.04^{D}	19.5±1.96	<.0001	
11d-TXB_2	7.46±1.07^{a}	6.88±1.17^{a}	6.37±1.71^{ba}	3.97±0.731^{b}	–^{c}	–^{c}	–^{c}	–^{c}	<.0001	
5-HETE	3174±303^{b}	4095±232^{a}	2334±249^{c}	2792±245^{cb}	1467±119^{d}	1679±152^{d}	826±95^{e}	893±70.2^{e}	<.0001	
5-oxoETE	524±86.6^{A}	690±60.9	374±63.1^{B}	442±99.6	225±42.2^{C}	241±43	153±32.6^{C}	208±72.8	<.0001	
5,15-DiHETE	152±22.5^{A}	178±17.3	130±22^{B}	114±15.3	92.7±5.1^{B}	93.8±10.8	46.2±5.16^{C}	50.4±8.45	<.0001	
8-HETE	200±24.7^{A}	230±18	170±24.7^{A}	228±30	106±10.4^{B}	111±13.6	67.9±17.2^{C}	74.4±8.67	<.0001	
9-HETE	882±31.3^{A}	1184±78.7	828±143^{A}	998±121	513±69.1^{B}	668±88.6	257±57.6^{C}	322±33.8	<.0001	0.0071
11-HETE	1004±141^{A}	971±152	670±115^{A}	916±170	398±40.8^{B}	446±95.7	265±36.1^{B}	289±34.2	<.0001	
12-HETE	1196±86.3^{A}	1588±198	1226±168^{A}	1561±202	694±127^{B}	797±103	403±68.3^{C}	415±51.6	<.0001	0.0388
12-oxoETE	35.0±6.95^{A}	40.1±8.03	26.0±4.66^{A}	40.6±11.7	17.4±3.55^{B}	19.0±5.48	15.6±4.79^{B}	20.5±8.17	0.0031	
tetranor 12-HETE	1.92±0.876^{b}	3.97±0.481^{a}	0.444±0.444^{cbd}	1.7±0.922^{cb}	0.335±0.335^{cd}	0.126±0.126^{d}	0.153±0.153^{d}	–^{d}	<.0001	
15-HETE	3296±330^{A}	3117±689	2167±422^{A}	3032±538	1350±232^{B}	1475±352	1073±128^{B}	1102±80.9	<.0001	
8,15-DiHETE	2493±301^{A}	2795±328	1745±294^{B}	1922±171	853±93.7^{C}	1024±110	553±45.1^{D}	628±54.9	<.0001	
15-oxoETE	275±62.0^{A}	421±98.8	208±41.1^{AB}	301±88.5	127±18.7^{BC}	183±35.8	110±21.7^{C}	169±34.5	0.0028	0.0355

HXB_3*	0.577±0.172^{A}	0.609±0.14	0.638±0.173^{A}	0.447±0.0439	0.214±0.0435^{B}	0.373±0.0713	0.147±0.043^{B}	0.225±0.0275	<.0001	
LTB_4	17.4±3.12^{A}	28.4±4.13	18.5±3.77^{A}	23.1±3.89	9.22±1.86^{B}	15±3.94	6.35±1.12^{C}	7.43±1.00	<.0001	0.0158
6t-LTB_4	–^{d}	–^{d}	–^{d}	–^{d}	12.9±4.2^{ba}	17.3±4.12^{a}	3.69±1.42^{dc}	7.01±2.18^{bc}	<.0001	
16-HETE	458±43.1^{A}	629±44.3	420±33.9^{A}	523±64.0	224±19.6^{B}	270±17.0	123±17.7^{C}	149±27.6	<.0001	0.0104
18-HETE	14.7±1.69	10.9±1.9	13.3±2.69	9.70±1.49	12.5±1.95	13.3±3.33	10.1±1.24	10.6±1.24		
20-HETE	77.3±25.5^{A}	37.5±7.73	27.0±2.8^{B}	33.1±5.34	25.9±3.48^{C}	15.5±2.17	26.9±5.86^{BC}	29.3±4.61	0.0022	
5,6-DiHETrE	56.4±10.8^{A}	63.0±12.5	53.5±11.3^{AB}	46.1±8.69	28.3±5.96^{B}	34.6±5.18	25.3±9.37^{C}	17.1±3.36	<.0001	
8,9-DiHETrE	19.4±3.40^{A}	19.8±3.06	12.7±1.55^{B}	14.2±2.41	6.58±0.951^{C}	9.76±1.23	3.67±0.591^{D}	3.85±0.641	<.0001	
11,12-DiHETrE	57.6±16.6^{A}	39.8±4.97	27.5±3.07^{B}	25±4.56	14.9±2.14^{C}	16.5±2.16	7.74±1.17^{D}	8.58±1.52	<.0001	
14,15-DiHETrE	39.8±6.02^{A}	41.0±4.47	31.3±4.05^{B}	30.5±3.9	17.9±2.03^{C}	21.2±3.03	10.8±1.43^{D}	11.9±2.10	<.0001	
5-iso $PGF_{2\alpha}$VI	202±35.0^{b}	295±21.9^{a}	138±14.8^{c}	138±10.9^{c}	107±16.4^{dc}	136±16.2^{c}	63.6±8.68^{d}	68.6±5.50^{d}	0.0453❖	
8-iso $PGF_{2\alpha}$III	170±17.9^{A}	231±20.5	113±20.3^{B}	120±13.2	79.1±17.4^{C}	84.7±12.4	43.8±4.49^{D}	46.7±3.04	<.0001	
8-iso 15k $PGF_{2\beta}$	14.6±2.04^{b}	28.8±4.09^{a}	–^{c}	–^{c}	–^{c}	–^{c}	4.87±3.29^{c}	–^{c}	0.0003	
Sum	17241±454^{A}	19735±677	12800±1700^{B}	15567±1580	7532±538^{C}	8706±904	5334±480^{D}	5435±491	<.0001	
LA derived oxylipins										
9-HODE	8525±854	9166±1551	9837±1461	12299±2492	7521±1043	6887±1337	7692±883	9865±923		
9-oxoODE	273±21.6	529±71.9	463±70.9	614±206	355±83.2	334±26.4	407±68.2	498±98.7		
13-HODE	4674±592^{B}	5741±838	6269±723^{A}	7597±1492	5133±491^{B}	4653±877	4113±510^{B}	5615±569	0.0493	
13-oxoODE	670±85.1	1426±292	1312±204	1779±546	864±190	1003±166	924±93.6	1755±248		0.0057
9,12,13-TriHOME	14013±6206	9482±878	20313±3620	13639±4106	12136±1842	10173±1441	14754±3686	10853±2354		
9,10-DiHOME	114±40.2	64.1±6.19	79.6±6.17	81.0±15.5	70.0±6.93	54.9±9.48	65.3±6.75	63.9±12		
12,13-DiHOME	80.8±20.7	80.4±12.7	91.4±11.5	82.9±11.9	88.0±8.4	62.5±7.44	76.5±12.6	66.8±12.3		
Sum	28349±5963^{B}	26489±2472	38365±4939^{A}	36092±6663	26168±1318^{B}	23168±3664	28031±3496^{AB}	28718±2802	0.0495	
EDA derived oxylipins										
15-oxoEDE	11.9±2.12^{A}	14.8±1.85	12.8±2.73^{A}	15.1±2.65	10.3±0.849^{B}	7.32±0.91	7.77±0.87^{AB}	12.7±2.78	0.0293	
GLA derived oxylipins										
13-HOTrE-γ	78.0±6.05^{A}	102±19.8	64.6±8.32^{A}	79.5±17.8	36.8±3.66^{B}	25.7±3.69	38.2±5.69^{B}	32.3±3.75	<.0001	
DGLA derived oxylipins										
PGE_1	21.6±6.22^{A}	31.2±3.08	22.8±4.48^{A}	24.7±2.73	10.3±1.87^{B}	12.3±1.67	5.84±0.851^{B}	5.97±0.791	<.0001	
$PGF_{1\alpha}$	40.2±2.22^{A}	48.1±4.54	20.2±2.72^{B}	25.5±1.45	15.8±3.43^{C}	14.6±2.26	15.8±1.50^{C}	15.2±1.08	<.0001	

8-HETrE	101±12.6^{cb}	177±8.94^{a}	116±13.4^{b}	158±21^{a}	74.1±9.77^{cd}	77.5±11.2^{cd}	62.7±5.3^{d}	60.8±5.55^{d}	0.0083	
15-HETrE	276±23.2^{A}	326±27.5	289±37.9^{A}	423±51.1	161±22.9^{B}	155±14.8	167±22.7^{B}	159±17.3	<.0001	0.0488
Sum	439±39.1^{A}	582±27.2	448±50.4^{A}	632±68.2	261±33.1^{B}	260±25.1	251±27.5^{B}	241±21.7	<.0001	0.0417
AdA derived oxylipins										
dihomo PGD_2*	0.0225±0.001^{A}	0.0405±0.00677	0.00997±0.00258^{B}	0.0166±0.00282	0.0145±0.00166^{B}	0.0127±0.00276	0.00542±0.00069^{C}	0.0061±0.00105	<.0001	
dihomo PGE_2*	0.0504±0.0242^{a}	0.0495±0.0145^{a}	–^{b}	–^{b}	–^{b}	–^{b}	–^{b}	0.00132±0.00083^{b}	<.0001	
dihomo $PGF_{2\alpha}$	55.9±15.6^{A}	66.7±9.21	17±2.70^{B}	23.2±2.76	7.69±1.39^{C}	7.11±1.15	6.28±0.861^{C}	6.33±0.907	<.0001	
Sum n-6	46175±6111^{A}	46990±2846	51707±6443^{A}	52408±7969	34016±1686^{B}	32174±4361	33669±3908^{B}	34445±2981	0.0002	
Sum n-3	12213±1003^{C}	7981±663	29602±3216^{B}	29785±4497	35569±3389^{B}	27879±4339	82623±11897^{A}	76281±9436	<.0001	0.0273
n6/n3 Ratio	3.92±0.452^{A}	6.27±0.611	1.78±0.119^{B}	1.84±0.156	0.994±0.081^{C}	1.22±0.1	0.452±0.064^{D}	0.489±0.049	<.0001	0.0088

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

# Denotes that oxylipin is not detected.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums. AA oxylipin sum includes AA non-enzymatic products.

Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 4.5. Kidney Phospholipid Fatty Acid In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
					ug/g tissue					
C140	104±8.91^{C}	136±13.6	187±11.5^{B}	144±32.4	242±14.7^{A}	190±45	198±14.3^{A}	247±21.0	<.0001	
C160	18378±1377^{A}	15823±804	15013±570^{B}	14098±1026	16552±1248^{A}	17053±812	16626±1308^{A}	16633±403	0.0378	
C161	527±104	618±125	634±65.4	453±71.9	–$^{\#}$	–	608±121	–		
C170	140±23.3^{C}	121±9.82	188±24.3^{BC}	176±54.4	265±46.2^{A}	214±16.6	244±41.8^{AB}	183±39.9	0.0062	
C180	16288±1205^{A}	15098±1269	13713±245^{B}	13163±909	15656±1951^{A}	17074±1283	14010±1458^{AB}	14844±593		
C181	10034±249^{a}	6891±689^{b}	6814±218^{cb}	4961±317^{d}	5634±567^{cd}	4875±168^{d}	5735±421^{CD}	5116±229^{d}	0.0082❖	
C182n6	7803±501^{C}	8268±638	9815±203^{B}	11163±911	10013±787^{B}	11446±383	12539±1537^{A}	14596±610	<.0001	0.0197
C183n3	99.1±8.8^{C}	63.6±12.1	969±59.4^{A}	764±75.3	147±19.2^{B}	87.1±12.5	125±13.8^{B}	96.4±3.9	<.0001	<.0001
C183n6	62.7±4.69	41.5±10	64.2±6.03	71.9±13.5	61.2±5.41	32.7±11.7	54.1±8.41	65.5±21.6		
C200	345±27.9	210±10.8	283±9.65	204±14.1	279±32.2	224±22.7	278±25.7	244±32.0		<.0001
C201	193±15.3^{a}	138±10.3^{b}	105±7.01^{c}	94.5±9.73^{dc}	79.3±5.66^{dc}	64.4±11^{d}	88.6±7.08^{dc}	63.9±12.8^{d}	<.0001	
C202n6	180±9.81^{A}	146±12.4	132±6.79^{B}	134±11.1	161±15.7^{AB}	140±9.21	157±19.9^{AB}	147±11.5		
C203n3	65.9±22.9^{BC}	72±15.3	292±16.7^{A}	232±19.0	105±11.6^{B}	90.6±11.3	61.5±13^{C}	59.9±14.4	<.0001	
C203n6	694±59.6^{A}	1057±102	679±69.6^{A}	937±80.6	588±67.9^{B}	731±27.0	743±82.7^{AB}	741±39.2	0.0323	0.0006
C204n6	27022±2088^{A}	25223±2181	14351±479^{B}	11492±2430	11121±1133^{C}	12654±508	7725±758^{D}	7765±340	<.0001	
C205n3	229±23.7^{D}	173±20.1	3446±283^{C}	2714±216	10073±1459^{A}	9878±492	7154±836^{B}	7742±708	<.0001	
C220	840±89.4^{A}	465±24.8	626±33^{B}	446±30.8	624±57.3^{B}	461±24.6	619±55.2^{B}	482±38.8		<.0001
C221	55.7±10.8	28.7±4.38	–	–	–	–	–	12.6±9.13		
C222n6	93.3±42.2	111±40.6	77.8±25.1	64.6±24	97.7±16.9	84.1±10.8	58.7±13.8	45.3±4.76		
C224n6	–^{A}	–	–^{B}	–	94.5±13.7^{C}	88.1±5.97	52.9±7.41^{D}	33.1±2.44	<.0001	0.0098
C225n3	242±26.8^{D}	209±21.0	1018±103^{B}	726±57.1	2830±387^{A}	2202±158	612±43.2^{C}	462±23.3	<.0001	0.0011
C225n6	302±45.5^{a}	259±46.9^{a}	–^{c}	–^{c}	–^{c}	–^{c}	88.9±9.23^{b}	–^{b}	<.0001	
C226n3	3800±352^{b}	1777±188^{d}	2671±208^{c}	1790±150^{d}	2389±230^{c}	1672±88.5^{d}	7589±610^{a}	6276±234^{a}	0.0089	
C240	3979±462	3253±224	3805±261	3516±291	4213±470	4139±255	4282±363	4014±309		
C241	1655±117^{A}	1421±79	949±54.3^{B}	874±85	744±60.8^{C}	677±25.9	786±79.8^{C}	662±50.6	<.0001	0.0274
n6/n3 Ratio	8.39±0.223^{A}	15.8±0.539	3.04±0.135^{B}	4.14±0.167	1.45±0.081^{C}	1.82±0.048	1.37±0.061^{C}	1.63±0.089	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.
# Denotes that oxylipin is not detected.
❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.
Values are mean±SE (n=5-6 for each), and based on dry tissue weight.

Table 4.6a. Liver N-3 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Female	Male	Diet	Sex
					ng/g tissue					
ALA derived oxylipins										
9-HOTrE	408±116^{C}	423±97.9	6261±2732^{A}	2684±355	604±149^{C}	358±49.2	910±251^{B}	1203±282	<.0001	
9-oxoOTrE	11.7±3.27^{C}	4.06±0.399	118±35.5^{A}	163±65.1	21.6±3.98^{B}	23.3±5.05	39.9±13.3^{B}	45.3±16.9	<.0001	
13-HOTrE	61.6±16.8^{B}	68.8±12.5	654±158^{A}	329±25.2	91.3±19.4^{B}	108±45	152±66^{B}	252±79.2	<.0001	
Sum	481±134^{C}	496±110	7033±2919^{A}	2674±542	717±156^{C}	489±92	1101±327^{B}	1501±361	<.0001	
EPA derived oxylipins										
PGE_3	19.4±2.76^{D}	54.7±7.41	306±50.3^{C}	403±78.6	1540±222^{A}	1923±425	471±91.7^{B}	748±113	<.0001	0.0004
TXB_3	–$^{\#c}$	–^{c}	7.90±2.13^{b}	6.40±0.707^{b}	16.6±2.40^{a}	15.3±2.26^{a}	4.83±0.715^{b}	6.19±0.885^{b}	<.0001	
5-HEPE	38.9±9.68^{D}	98.6±28.1	554±79.5^{C}	870±244	5164±1031^{A}	8718±2850	1199±237^{B}	2146±337	<.0001	0.0017
8-HEPE	12.7±2.91^{D}	16.3±2.17	194±54.3^{C}	176±61.6	826±156^{A}	1004±281	253±75.1^{B}	354±85	<.0001	
9-HEPE	21.7±4.9^{D}	35.5±7.08	271±62.4^{C}	265±99.8	1922±392^{A}	2091±692	509±135^{B}	733±199	<.0001	
12-HEPE	196±50.6^{C}	165±33.2	5755±3355^{B}	2836±1012	8903±2591^{A}	5971±1097	5392±1199^{AB}	5051±1374	<.0001	
15-HEPE	110±28.5^{C}	94.9±11.9	2786±1534^{B}	1987±857	3972±1120^{A}	3529±519	3074±1098^{AB}	2955±844	<.0001	
18-HEPE	35.5±10.4^{D}	73.6±22.1	654.9±170.6^{C}	620.9±229.6	4582.5±943.6^{A}	5135.3±1625.2	1384.9±323.8^{B}	1922.9±613.9	<.0001	
RvE_1	35.5±10.4^{D}	73.6±22.1	655±171^{C}	621±230	4582±944^{A}	5135±1625	1385±324^{B}	1923±614	<.0001	
Sum	441±105^{D}	552±106	5351±599^{C}	7171±2564	26948±5557^{A}	28422±5852	12300±2907^{B}	13928±3228	<.0001	
DHA derived oxylipins										
4-HDoHE	668±171^{B}	1005±255	489±133^{B}	757±257	753±165^{B}	1593±654	4793±1781^{A}	15793±7078	<.0001	0.0132
7-HDoHE	184±60.6^{B}	150±11.6	187±55.4^{B}	109±10.2	163±32^{B}	238±99	1596±649^{A}	1740±412	<.0001	
8-HDoHE	270±82.8^{B}	271±31.6	222±76.4^{B}	350±175	249±44.6^{B}	359±120	2028±553^{A}	3993±1343	<.0001	
10-HDoHE	136±35.1^{B}	130±13.4	160±39.2^{B}	167±82	217±64.8^{B}	296±134	1140±321^{A}	2268±771	<.0001	
11-HDoHE	271±74.5^{B}	326±47.3	255±70^{B}	343±189	315±68.5^{B}	472±200	2223±686^{A}	4897±1789	<.0001	
13-HDoHE	754±175^{B}	520±89.3	1232±649^{B}	996±528	767±153^{B}	714±210	6935±1721^{A}	9748±3011	<.0001	
14-HDoHE	1327±407^{B}	879±141	2386±1380^{B}	1882±1051	971±263^{B}	949±202	12220±3199^{A}	16202±5311	<.0001	
16-HDoHE	249±68.1^{B}	219±30.4	363±120^{B}	360±176	322±69.2^{B}	353±121	1990±649^{A}	2877±1073	<.0001	
17-HDoHE	944±259^{B}	963±119	1501±500^{B}	1752±961	1434±326^{B}	1804±656	9726±3286^{A}	14113±7207	<.0001	

19,20-DiHDoPE	110±25.1^{B}	346±50.8	119±23.5^{B}	379±102	76.5±11.6^{B}	335±80.6	556±103^{A}	2035±432	<.0001	<.0001
19,20-EpDPE	18.2±4.88^{B}	21.8±4.76	13.6±5.95^{B}	15.7±8.29	12.9±2.98^{B}	11.9±1.5	107±36.8^{A}	190±96	<.0001	
20-HDoHE	928±291^{B}	1198±211	1166±366^{B}	1485±733	1321±257^{B}	1872±528	7992±2107^{A}	11462±3504	<.0001	
Sum	5859±1534^{B}	6027±936	8114±3380^{B}	8596±4235	6613±1263^{B}	9010±2952	51410±14042^{A}	85132±30288	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicated differences between diets.
# Denotes that oxylipin is not detected.
Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 4.6b. Liver N-6 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Female	Male	Diet	Sex
					ng/g tissue					
AA derived oxylipins										
PGA_2	33.4±10.4^{a}	20.2±13.1^{ba}	18.6±7.74^{bac}	10.9±5.93^{bc}	–$^{\#\ c}$	–^{c}	–^{c}	–^{c}	0.0006	
PGD_2	354±84.2^{A}	533±71.3	143±25.7^{BC}	129±21.0	214±38.1^{B}	218±54.3	95.9±27.0^{C}	118±36.9	<.0001	
15deoxy-PGD_2	21.4±6.47^{AB}	34±3.92	21.8±7.21^{B}	16.3±4.4	32.8±7^{A}	23.3±2.89	19.6±2.06^{AB}	20.2±7.57		
PGE_2	166±34.4^{A}	247±29.5	59±7.74^{BC}	52.2±6.94	86.8±15.2^{B}	91.5±18.7	38.4±7.57^{C}	48.1±10.9	<.0001	
bicyclo PGE_2	44.1±33.6^{B}	10.6±4.25	26.8±8.19^{A}	27.7±8.33	41.9±9.7^{A}	23±4.08	34.4±11.1^{A}	32.3±7.98	0.0138	
6k-$PGF_{1\alpha}$	8.04±0.73^{A}	9.00±0.403	4.26±0.612^{A}	5.38±0.607	3.77±0.433^{A}	4.66±0.387	3.05±0.312^{B}	4.47±0.66	<.0001	0.0064
$PGF_{2\alpha}$	242±49.9^{A}	186±15.4	78.4±10.6^{B}	36.2±3.19	41.7±7.11^{C}	35.1±6.9	37.7±7.29^{C}	29.4±9.66	<.0001	0.0056
15deoxy-PGJ_2	43.4±11.3^{A}	36.9±21.1	–^{B}	–	–^{B}	–	–^{B}	–	<.0001	
TXB_2	27.2±3.76^{A}	36.8±4.77	13.1±3.81^{B}	8.68±1.11	5.26±0.865^{C}	5.79±0.708	2.86±0.636^{D}	2.88±1.07	<.0001	
8-HETE	177±48.5^{A}	151±24.1	70.4±18.6^{B}	47.4±16	56.5±11.9^{B}	51.3±16.4	55.8±17.8^{B}	56.2±26.7	0.0002	
9-HETE	637±200^{A}	746±126	261±60.6^{B}	202±66.5	297±53.8^{B}	295±91.4	232±65.3^{B}	266±113	0.0006	
11-HETE	461±158^{A}	457±81.2	148±23.3^{B}	108±21.7	310±123^{AB}	318±146	148±28.5^{B}	173±62.7	0.004	
5-HETE	1021±300^{A}	1377±176	414±79.9^{B}	570±103	486±65^{B}	623±124	358±73.7^{B}	447±155	<.0001	
5-oxoETE	135±33.9^{A}	87.8±9.63	51.3±14^{B}	46.2±10.2	62.4±8.2^{B}	53.1±8.28	48.5±14.9^{B}	102±73.4	0.0069	
12-HETE	3891±1122^{A}	2892±614	2358±1272^{B}	1179±361	686±140^{C}	457±79.2	1525±410^{B}	1023±366	<.0001	
12-oxoETE	24.6±4.85^{a}	16.8±2.24^{ba}	7.29±2.16^{bc}	4.79±1.35^{bc}	8.06±2.34^{bc}	–^{c}	10.1±3.91^{bc}	16.4±10.7^{ba}	0.0031	
tetranor 12-HETE	11.5±2.33^{a}	3.24±0.84^{cb}	6.83±2.81^{b}	1.52±0.696^{c}	1.17±0.457^{c}	1.15±0.641^{c}	2.96±0.781^{cb}	1.62±0.710^{c}	0.0231❖	
15-HETE	1795±503^{A}	2471±602	1100±269^{B}	705±171	1187±180^{B}	983±264	960±149^{B}	810±295	0.0016	
15-oxoETE	40.9±12.8^{A}	30.3±4.64	18.7±3.01^{B}	16.1±2.79	40±12.8^{A}	50.4±20.6	26.8±5.44^{AB}	26.8±8.24	0.0332	
8,15-DiHETE	494±133	731±83.7	670±115	655±226	429±54.9	611±187	386±96.2	397±68.0		
5,6-DiHETrE	87.7±31.8^{A}	199±41.4	27.2±7.57^{B}	67.8±16.8	20±1.53^{BC}	55.1±10.1	16.1±2.69^{C}	40.1±12.8	<.0001	<.0001
8,9-EpETrE	3.89±2.13^{A}	7.38±1.94	4.45±1.73^{AB}	2.51±1.09	6.06±0.776^{AB}	2.65±0.319	2.88±0.546^{B}	3.45±1.99		
8,9-DiHETrE	35.2±7.41^{A}	289±47.9	21±3.25^{B}	97.7±17.7	15.2±1.56^{B}	86.7±14.1	9.67±0.929^{C}	37.4±7.6	<.0001	<.0001
11,12-DiHETrE	115±23.9^{A}	831±144	63.8±9.58^{B}	235±39.8	40.8±5.53^{B}	212±43.3	26.5±1.81^{C}	98.5±20.1	<.0001	<.0001
14,15-DiHETrE	101±22.5^{A}	917±198	59.9±8.7^{B}	231±43.4	40.0±5.02^{B}	230±64.4	24.2±2.47^{C}	109±22.8	<.0001	<.0001

16-HETE	3689±534^{A}	3080±390	7139±2438^{A}	2903±368	1472±371^{B}	1080±176	2133±419^{B}	1310±287	<.0001	0.0099
17-HETE	54.5±4.44	52.9±7.71	64.7±22.6	57.8±6.93	47.6±10.2	54.2±8.68	42.5±6.78	44.6±6.34		
18-HETE	22.4±5.63^{b}	46.7±11.7^{b}	14.1±3.67^{b}	22.8±2.58^{b}	72.3±22.1^{b}	195±73.9^{a}	17.7±5.5^{b}	59.8±7.12^{b}	0.0006	
19-HETE	115±22.9^{A}	430±89.7	66.2±11.5^{BC}	126±18.6	44.7±4.13^{B}	254±76.3	29.5±4.11^{C}	120±7.84	<.0001	<.0001
20-HETE	45.5±9.54^{A}	55.1±10.8	–^{B}	–	–^{B}	–	–^{B}	–	<.0001	
5-iso $PGF_{2\alpha}$VI	37.8±10.1^{A}	48.1±5.36	14.3±2.21^{B}	22.2±2.47	18.7±2.45^{B}	27.7±4.57	20±3.11^{B}	22.3±6.06	0.0001	0.0202
8-iso $PGF_{2\alpha}$III	28.2±6.31^{A}	34±4.04	12.3±1.58^{B}	9.86±1.38	12.7±1.99^{B}	15.3±3.6	12.4±2.53^{B}	11.1±2.97	<.0001	
Sum	13962±2882^{A}	16064±2229	12958±4194^{B}	7596±1286	5779±418^{C}	6059±1071	6319±921^{C}	5425±1241	<.0001	
LA derived oxylipins										
9-HODE	3698±1008^{BC}	5420±1070	3659±1237^{C}	3430±1161	7357±1038^{AB}	6582±2161	6504±1403^{A}	9052±2273	0.0231	
9-oxoODE	89.8±24.2^{B}	45.1±6.57	86±26.9^{B}	120±43.7	188±40.6^{A}	228±64.4	285±59.1^{A}	394±140	<.0001	
13-HODE	1610±452^{BC}	2651±647	1574±436^{C}	1639±704	3015±601^{AB}	2918±1002	3423±957^{A}	3794±926	0.028	
13-oxoODE	101±33.7^{B}	83.8±16.4	117±25.2^{B}	147±38.4	491±203^{A}	679±349	398±113^{A}	687±221	<.0001	
9,12,13-TriHOME	4570±1086^{C}	2017±357	10642±4994^{AB}	6057±1857	5385±890B^{C}	3185±559	10914±3259^{A}	9946±2962	0.0005	0.0213
12,13-EpOME	23.0±7.1^{AB}	28.5±7.26	11.8±3.26^{B}	13.7±6.24	27.6±10.8^{AB}	18.0±4.38	35.0±11.1^{A}	32.0±8.67	0.0264	
12,13-DiHOME	159±37.7	705±145	155±28.3	405±93.5	149±24.3	550±176	153±12.5	391±78.5		<.0001
9,10-EpOME	28.7±8.68^{AB}	35.8±9.6	33.2±20.1^{B}	16.9±7.68	59.3±20.1^{A}	26.9±5.02	43.4±10.7^{A}	35.6±13.1		
9,10-DiHOME	348±103	931±145	448±82.5	523±135	396±99.5	714±213	356±41.1	515±105		0.0026
Sum	10628±2199^{B}	11917±1970	16726±6755^{B}	12351±3932	17068±1606^{AB}	14901±3772	22110±5465^{A}	24846±5933	0.0203	
EDA derived oxylipins										
15-oxoEDE	1.96±0.569^{B}	2.51±0.597	2.03±0.37^{B}	3.33±1.1	3.51±0.407^{A}	6.11±1.66	4.96±1.29^{A}	11.0±2.91	<.0001	0.0141
GLA derived oxylipins										
13-HOTrE-γ	77.2±20.3^{A}	94.4±16.3	36.8±9.82^{B}	31.9±13.1	37.4±8.1^{B}	23.5±6.62	37.9±8.95^{B}	54.3±16.8	0.0005	
DGLA derived oxylipins										
$PGF_{1\alpha}$	16.3±4.89^{a}	15.4±3.47^{a}	3.91±2.48^{b}	–^{b}	17.3±1.45^{a}	2.0±2.0^{b}	2.98±2.98^{b}	1.34±1.34^{b}	0.0003	
5-HETrE	41.5±8.83^{b}	149±21.8^{a}	7.65±3.81^{d}	38.3±11.5^{cb}	6.1±2.04^{d}	15.5±1.5^{cbd}	7.73±1.85^{d}	11.4±4.44^{cd}	<.0001	
8-HETrE	36±9.29^{AB}	96±27.4	24.3±4.48^{B}	43.5±8.39	43.2±8.44^{AB}	57±17.4	57.1±14^{A}	80.5±24.9		0.0154
15-HETrE	647±128^{A}	958±243	561±225^{A}	558±128	298±66.7^{B}	310±44.4	793±162^{A}	599±131	0.0012	
Sum	741±145^{A}	1218±280	597±231^{BC}	640±146	365±67.4^{C}	385±55.0	861±173^{AB}	692±151	0.0013	
AdA derived oxylipins										

dihomo PGD_2*	0.002±0.002^{bc}	0.008±0.005^{ba}	–^{c}	–^{c}	–^{c}	–^{c}	0.002±0.001^{bc}	0.01±0.002^{a}	0.0065	
dihomo $PGF_{2\alpha}$	24.7±3.97^{A}	22.6±4.77	6.14±0.92^{B}	–	–^{B}	–	–^{B}	–	<.0001	
Sum n-6	25435±4939	29319±4222	30326±11160	20622±5288	23253±1959	21374±4851	29333±6398	31028±7270		
Sum n-3	6781±1732^{D}	7075±1106	25681±11445^{C}	18441±6372	34278±6782^{B}	37921±8749	64811±17037^{A}	100561±33599	<.0001	
n6/n3 Ratio	4.14±0.396^{A}	4.21±0.153	1.31±0.1^{B}	1.23±0.08	0.77±0.1^{C}	0.574±0.035	0.537±0.111^{D}	0.353±0.035	<.0001	0.0362

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

# Denotes that oxylipin is not detected.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums. AA oxylipin sum includes AA non-enzymatic products.

Values are mean±SE (n=5-6 for each), and are based on dry tissue weight.

Table 4.7 Liver Phospholipid Fatty Acid In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
					ug/g tissue					
C140	68.4±16.8^{d}	134±9.53B^{a}	113±11.0^{bac}	92.8±7.26^{dc}	101±16.3^{bdc}	144±16.3^{a}	85.2±5.24^{dc}	129±12.0^{ba}	0.0122❖	
C160	7698±527^{e}	9709±625^{d}	8545±237^{ed}	11351±698^{c}	8303±241^{ed}	13017±808^{b}	8537±123^{ed}	15656±431^{a}	0.0001	
C161	310±30.9^{cd}	466±101^{bc}	348±70.8^{bcd}	519±55^{ba}	239±36^{d}	657±84.6^{a}	219±19^{d}	683±70.3^{a}	0.0346	
C170	113±22.2^{bc}	69.7±13.2^{c}	173±18.6^{a}	83±20.8^{bc}	129±12.5^{ba}	101±13.8^{bc}	102±13.6^{bc}	126±16.8^{ba}	0.0173	
C180	20612±4601	15148±1707	25875±1729	16007±1787	19692±1004	13002±1166	21618±2385	15352±1890		0.0001
C181	1928±57.5^{A}	2678±181	1802±163^{AB}	2365±157	1390±70.7^{B}	2466±233	1405±114^{B}	2255±92.7	0.0025	<.0001
C182n6	4064±217^{e}	4648±280^{e}	8497±488^{ba}	8526±597^{b}	5717±489^{d}	6874±527^{c}	7048±387^{c}	10145±333^{a}	0.0395	
C183n3	27.6±11.0^{d}	30.9±6.16^{d}	786±85.3^{a}	376±27^{b}	76.0±14.0^{c}	73.4±13.4^{c}	54.4±10.3^{dc}	82.3±13.5^{c}	0.0312	
C183n6	39.0±9.39	129±21.6	59.6±14.2	105±25.5	57.2±15.2	102±15.4	34.7±5.91	65±17.9		<.0001
C200	71.3±7.94	62±7.61	71.1±8.1	111±47.6	128±27.7	56.4±10.5	80.7±31.9	92.7±42.4		
C201	90.6±10.4	111±8.1	249±187	173±74.7	287±114	50.9±13.6	304±138	174±96.1		
C202n6	166±29.1	155±12.2	177±26.9	212±27.4	352±121	175±14.7	318±88.9	223±24.9		
C203n3	50.3±18^{C}	18.3±7.73	335±13^{A}	300±31.6	402±193^{B}	67.8±17.0	353±255^{B}	59.9±24.8	<.0001	0.016
C203n6	651±44.1	1501±207	974±76.7	1963±220	1306±287	1355±266	1150±260	1510±153		0.0001
C204n6	18705±726^{A}	15177±635	13429±776^{B}	10212±583	7948±606^{C}	7006±758	4166±269^{D}	3465±332	<.0001	0.0002
C205n3	251±65.5^{C}	190±39	7456±963^{B}	4457±421	9673±946^{A}	6872±655	5801±582^{B}	4471±623	<.0001	0.0004
C220	351±57.9	290±39.9	299±28.2	340±45.8	319±30.4	235±39.6	282±35.4	291±47.8		
C221	34.9±19.8	205±84	4.65±3.1	116±69.8	27.2±23.1	77.1±54.2	1.41±0.897	49.0±47.8		0.0091
C222n6	326±198B^{a}	246±120B^{a}	588±250^{a}	3.81±2.57^{b}	11.7±11.7^{b}	67.8±67.8^{b}	–^{b}	50.3±50.3^{b}	0.0311	
C224n6	300±55.7^{a}	260±18^{a}	36.5±9.08^{c}	244±83.5^{ba}	13.8±8.09^{c}	119±57.8^{bc}	4.73±2.3^{c}	57.3±41.6^{c}	<.0001	
C225n3	198±33^{D}	364±51.5	1356±90.1^{B}	1647±218	2874±283^{A}	4225±503	574±44.2^{C}	995±162	<.0001	<.0001
C225n6	871±136^{a}	799±69.5^{a}	159±71.1^{cb}	250±92.6^{cb}	80.8±55.2^{c}	160±57.8^{cb}	160±42.5^{cb}	380±78.3^{b}	0.0169	
C226n3	8199±938^{b}	4638±257^{c}	9327±1349^{b}	7548±974^{b}	4975±576^{c}	5033±466^{c}	12618±518^{a}	15713±735^{a}	0.0013	
C240	808±100^{BC}	411±19.9	940±59.1^{AB}	525±41.5	656±26.9^{C}	480±40.3	887±94.2^{A}	630±48.9	0.0088	<.0001
C241	521±63.9^{A}	259±14.4	423±40.6^{AB}	366±79.7	201±17.0^{B}	172±17.5	241±23.0^{B}	179±15.6	<.0001	0.0001

n6/n3 Ratio	3.27±0.0690^{A}	4.34±0.235	1.29±0.103^{B}	1.64±0.117	0.882±0.066^{C}	1.04±0.085	0.642±0.049^{D}	0.729±0.057	<.0001	0.0003

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

Values are mean±SE (n=5-6 for each), and based on dry tissue weight.

Table 4.8a. Serum N-3 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Female	Male	Diet	Sex
					ng/ml					
ALA derived oxylipins										
9-HOTrE	0.527±0.0947^{B}	0.833±0.129	8.03±0.923^{A}	8.67±1.36	0.836±0.171^{B}	0.801±0.221	0.499±0.0611^{B}	1.92±0.938	<.0001	0.0383
9-oxoOTrE	0.121±0.0144^{B}	0.0974±0.0205	1.17±0.141^{A}	0.873±0.245	0.0938±0.0218^{B}	0.0526±0.00805	0.0605±0.0135^{B}	0.141±0.0868	<.0001	
13-HOTrE	0.946±0.205^{B}	1.27±0.215	11.2±1.05^{A}	11.7±1.26	1.19±0.22^{B}	0.959±0.204	0.623±0.0839^{B}	1.33±0.36	<.0001	
12,13-DiHODE	0.123±0.0261^{B}	0.105±0.0261	1.06±0.136^{A}	0.987±0.113	0.203±0.0427^{B}	0.137±0.0523	0.108±0.0164^{B}	0.134±0.0452	<.0001	
Sum	1.72±0.321^{B}	2.30±0.38	21.5±1.94^{A}	22.2±2.67	2.32±0.439^{B}	1.94±0.466	1.29±0.138^{B}	3.50±1.33	<.0001	
EPA derived oxylipins										
PGE_3	–$^{\#b}$	–^{b}	0.075±0.0105^{b}	0.172±0.0424^{b}	0.406±0.216^{b}	1.41±0.526^{a}	0.0863±0.0202^{b}	0.222±0.0582^{b}	0.0002	
$PGF_{3\alpha}$	0.212±0.0616^{B}	0.0406±0.0105	0.2±0.0311^{AB}	0.0686±0.0209	0.268±0.0396^{A}	0.11±0.0358	0.179±0.0313^{B}	0.0497±0.00899		<.0001
TXB_3	0.0429±0.00922^{D}	0.104±0.0218	0.479±0.0774^{B}	0.928±0.187	2.26±0.88^{A}	2.77±0.814	0.385±0.0785^{C}	0.487±0.154	<.0001	0.0042
5-HEPE	0.0575±0.00951^{D}	0.141±0.0207	0.67±0.111^{C}	1.02±0.11	5.24±1.23^{A}	7.33±1.43	1.13±0.182^{B}	2.7±0.862	<.0001	<.0001
8-HEPE	0.0301±0.00457^{D}	0.0402±0.00574	0.235±0.0468^{C}	0.277±0.0217	0.974±0.179^{A}	1.15±0.122	0.278±0.0382^{B}	0.481±0.114	<.0001	0.0087
12-HEPE	1.93±0.400^{C}	5.04±1.20	47.8±10.4^{B}	55.4±8.16	331±99.1^{A}	322±49.5	40.6±6.28^{B}	65.1±14.6	<.0001	0.0115
15-HEPE	0.0605±0.00748^{C}	0.155±0.0253	1.01±0.194^{B}	1.77±0.302	5.58±1.31^{A}	7.48±0.843	1.26±0.127^{B}	2.10±0.38	<.0001	<.0001
18-HEPE	0.0614±0.00984^{D}	0.118±0.0241	1.73±0.414^{C}	1.83±0.292	9.23±1.75^{A}	14.2±2.56	1.84±0.241^{B}	4.10±1.19	<.0001	0.0026
Sum	2.4±0.458^{C}	5.64±1.25	52.2±11.1^{B}	61.4±8.49	354±103^{A}	356±54.3	45.8±6.77^{B}	75.3±16.3	<.0001	0.0093
DHA derived oxylipins										
4-HDoHE	1.02±0.155^{B}	1.07±0.15	1.17±0.208^{B}	1.62±0.227	0.878±0.216^{B}	1.01±0.148	7.33±1.48^{A}	37.1±19.8	<.0001	0.0129
7-HDoHE	0.203±0.0158^{C}	0.156±0.0238	0.221±0.0388^{B}	0.299±0.0504	0.216±0.036^{BC}	0.218±0.0225	2.28±0.32^{A}	4.56±1.47	<.0001	
8-HDoHE	0.253±0.0346^{B}	0.243±0.0518	0.292±0.0831^{B}	0.343±0.0628	0.228±0.0328^{B}	0.265±0.0593	3.89±0.426^{A}	6.8±2.16	<.0001	
10-HDoHE	0.246±0.0272^{C}	0.247±0.025	0.263±0.0319^{B}	0.521±0.0793	0.405±0.0762^{B}	0.442±0.0681	2.47±0.367^{A}	3.14±0.337	<.0001	0.013
11-HDoHE	0.104±0.013^{C}	0.131±0.021	0.218±0.0402^{B}	0.323±0.0528	0.214±0.0408^{B}	0.254±0.0327	1.88±0.268^{A}	4.54±1.7	<.0001	0.0042
13-HDoHE	0.456±0.0367^{C}	0.511±0.0519	0.578±0.105^{B}	0.947±0.119	0.61±0.126^{BC}	0.626±0.0749	4.05±0.543^{A}	7.24±1.92	<.0001	0.0067
14-HDoHE	4.05±0.675^{C}	5.1±0.86	5.97±1.58^{BC}	7.8±1.1	7.38±1.8^{B}	7.5±1.28	25±3.12^{A}	66.5±28	<.0001	0.0232
16-HDoHE	0.284±0.0232^{C}	0.303±0.0361	0.352±0.0518^{B}	0.489±0.0566	0.29±0.053^{C}	0.307±0.0353	2.78±0.37^{A}	5.06±1.32	<.0001	0.0192
17-HDoHE	3.32±0.199^{B}	4.93±0.716	3.56±0.646^{B}	7.65±1.44	3.83±0.463^{B}	4.41±0.535	20.3±2.39^{A}	46.4±10.2	<.0001	<.0001

16,17-EpDPE	0.0883±0.0214^{d}	0.152±0.0234^{dc}	0.17±0.0319^{dc}	0.23±0.0317^{c}	0.136±0.0463^{dc}	0.224±0.0449^{c}	0.717±0.131^{b}	3.48±1.26^{a}	0.0145❖	
19,20-EpDPE	0.0849±0.0103^{dc}	0.0849±0.00948^{dc}	0.128±0.0179^{c}	0.101±0.0147^{dc}	0.0704±0.0161^{d}	0.0869±0.0127^{dc}	0.535±0.0695^{b}	1.58±0.482^{a}	0.0261	
19,20-DiHDoPE	0.52±0.0689^{C}	0.468±0.0726	0.632±0.118^{B}	1.21±0.238	0.383±0.0376^{C}	0.584±0.0982	3.4±0.245^{A}	5.36±1.05	<.0001	0.0044
20-HDoHE	0.488±0.0602^{C}	0.573±0.117	1.06±0.317^{B}	1.12±0.212	0.766±0.156^{B}	0.858±0.074	7.57±1.19^{A}	19.2±5.87	<.0001	0.0393
Sum	11.1±0.689^{C}	14.0±1.68	14.6±3.11^{B}	22.7±3.29	15.4±2.7^{BC}	16.8±2.12	82.2±9.5^{A}	140±21.0	<.0001	0.0017

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

# Denotes that oxylipin is not detected.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums.

Values are mean±SE (n=5-6 for each), and are based on ng/ml serum.

Table 4.8b. Serum N-6 Oxylipins In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	Female	Male	Female	Male	Female	Male	Female	Male	Diet	Sex
					ng/ml					
AA derived oxylipins										
PGD_2	2.18±0.589^{A}	4.43±1.27	1.17±0.209^{BA}	2.44±0.527	0.941±0.238^{B}	1.46±0.443	0.407±0.133^{C}	0.942±0.506	<.0001	0.0043
PGE_2	3.64±0.963^{A}	7.63±2.77	2.11±0.269^{A}	3.93±0.781	1.41±0.483^{B}	2.09±0.727	0.705±0.257^{C}	0.957±0.374	<.0001	0.0299
15k-PGE_2	0.0232±0.00723^{B}	0.0821±0.0321	0.0338±0.00719^{B}	0.0723±0.0199	0.122±0.0279^{A}	0.157±0.0651	0.0295±0.00415^{B}	0.122±0.0653	0.0061	0.0166
6k-$PGF_{1\alpha}$	0.0865±0.0236^{A}	0.108±0.0154	0.0459±0.00819^{B}	0.0655±0.00743	0.0517±0.0111^{B}	0.0532±0.0183	0.05±0.0101^{B}	0.0436±0.0105	0.0062	0.4032
$PGF_{2\alpha}$	1.19±0.29^{A}	2.91±1.04	0.867±0.0914^{A}	1.55±0.322	0.567±0.188^{B}	0.862±0.248	0.265±0.052^{C}	0.47±0.156	<.0001	0.0045
PGJ_2	0.63±0.163^{b}	2.85±1.25^{a}	–$^{\#b}$	–^{b}	–^{b}	–^{b}	–^{b}	–^{b}	0.0378❖	
15deoxy-PGJ_2	0.252±0.046^{ba}	0.285±0.0562^{ba}	0.323±0.0878^{a}	0.424±0.1^{a}	326±0.782^{a}	0.949±0.949^{bc}	402±0.468^{a}	–^{c}	0.0031	
TXB_2	29.7±9.9^{A}	70.1±22.1	18.7±1.83^{A}	37.9±7.07	14.3±4.99^{B}	14.3±3.23	4.16±0.625^{C}	5.31±1.32	<.0001	0.0103
5-HETE	2.09±0.169^{A}	3.62±0.651	1.19±0.111^{B}	1.97±0.149	1.1±0.246^{B}	1.57±0.203	0.5±0.0917^{C}	1.23±0.393	<.0001	<.0001
5-oxoETE	0.826±0.0808^{A}	1.07±0.215	0.598±0.105^{AB}	0.635±0.0822	0.493±0.142^{B}	0.587±0.197	0.136±0.0167^{C}	0.488±0.286	<.0001	
5,15-DiHETE	0.0241±0.00361^{C}	0.036±0.00805	0.0547±0.0111^{B}	0.131±0.0198	0.35±0.0621^{A}	0.355±0.0601	0.0819±0.0134^{B}	0.156±0.0297	<.0001	0.0008
8-HETE	0.504±0.0913^{ab}	0.709±0.094^{a}	0.372±0.0677^{bcd}	0.549±0.0472^{ab}	0.427±0.109^{bc}	0.546±0.0775^{ab}	0.164±0.0362^{d}	0.255±0.0673^{cd}	<.0001	
9-HETE	1.19±0.148^{A}	1.7±0.289	0.786±0.162^{B}	1.11±0.0773	0.861±0.161^{B}	1.03±0.210	0.373±0.073^{C}	0.597±0.135	<.0001	0.0152
11-HETE	8.07±2.18^{A}	15.5±3.61	5.79±0.873^{BA}	10.5±1.6	5.26±1.58^{B}	6.98±2.03	2.03±0.711^{C}	2.55±0.574	<.0001	0.0059
12-HETE	121±30.9^{A}	175±29	98.4±20.2^{A}	126±16.0	124±31.9^{A}	136±28.1	45.8±9.13^{B}	60.7±14.2	<.0001	
12-oxoETE	1.80±0.618^{A}	0.900±0.129	0.876±0.265^{A}	1.03±0.158	0.353±0.116^{B}	0.535±0.0855	0.489±0.193^{B}	0.487±0.158	0.0001	
tetranor 12-HETE	0.141±0.0384^{A}	0.128±0.0197	0.0988±0.0222^{B}	0.079±0.0117	0.0995±0.0166^{AB}	0.0946±0.0179	0.0528±0.00705^{C}	0.0647±0.0194	0.0017	
15-HETE	7.77±1.8^{A}	15.3±3.55	5.05±0.666^{A}	8.94±1.09	3.87±1.1^{B}	5.3±1.32	1.74±0.474^{C}	2.89±0.571	<.0001	0.0005
15-oxoETE	0.7±0.259^{A}	0.657±0.124	0.347±0.0537^{AB}	0.448±0.0519	0.315±0.0719^{B}	0.404±0.0838	0.139±0.0545^{C}	0.317±0.18	<.0001	
8,15-DiHETE	–^{c}	0.0962±0.0962^{bc}	0.671±0.426^{a}	–^{c}	0.113±0.113^{bc}	0.36±0.125^{bac}	–^{c}	0.51±0.136^{ba}	0.0097	
HXB_3*	0.694±0.161^{A}	1.09±0.184	0.769±0.137^{A}	0.806±0.0834	0.700±0.124^{A}	0.702±0.101	0.314±0.0513^{B}	0.441±0.0955	0.0002	
LTB_4	0.0255±0.00455^{b}	0.0452±0.00863^{a}	0.011±0.00251^{cbd}	0.0406±0.00536^{a}	–^{d}	–^{d}	0.00731±0.00183^{cd}	0.0214±0.00924^{cb}	<.0001	
5,6-DiHETrE	0.385±0.0538^{A}	1.23±0.337	0.300±0.0636^{B}	0.717±0.109	0.226±0.0407^{B}	0.473±0.059	0.113±0.0167^{C}	0.251±0.0343	<.0001	<.0001
8,9-EpETrE	0.0287±0.0146	0.0676±0.0332	0.0634±0.0113	0.0222±0.0141	0.0249±0.00876	0.0318±0.0111	0.0159±0.00523	0.0435±0.0143		
8,9-DiHETrE	0.0418±0.00432^{A}	0.0631±0.0076	0.0288±0.00269^{A}	0.058±0.00736	0.025±0.00274^{B}	0.0393±0.00497	0.0112±0.00248^{C}	0.0227±0.00407	<.0001	<.0001

11,12-EpETrE	0.0686±0.0104^{A}	0.150±0.035	0.0475±0.00557^{B}	0.0764±0.0118	0.029±0.00508^{C}	0.0615±0.0121	0.0168±0.00329^{D}	0.0249±0.00513	<.0001	<.0001
11,12-DiHETrE	0.162±0.0195^{A}	0.355±0.052	0.142±0.0138^{AB}	0.322±0.0695	0.117±0.00899^{B}	0.211±0.0391	0.0637±0.0128^{C}	0.119±0.0251	<.0001	<.0001
14,15-EpETrE	0.168±0.0141^{A}	0.337±0.0845	0.150±0.0194^{B}	0.153±0.0175	0.118±0.0208^{B}	0.173±0.0291	0.0424±0.00664^{C}	0.107±0.02	<.0001	0.0003
14,15-DiHETrE	0.364±0.0253^{A}	0.617±0.0714	0.277±0.0262^{A}	0.556±0.106	0.219±0.0202^{B}	0.316±0.0385	0.123±0.0194^{C}	0.186±0.0367	<.0001	<.0001
17,18-EpETE	–^{c}	–^{c}	0.137±0.0259^{c}	0.0972±0.03^{c}	0.790±165^{b}	1.44±0.399^{a}	0.0933±0.018^{c}	0.222±0.0592^{c}	<.0001	
17-HETE	0.0633±0.00482^{B}	0.0609±0.0107	0.0914±0.0079^{A}	0.121±0.0133	0.131±0.0148^{A}	0.142±0.0254	0.0777±0.0133^{B}	0.0903±0.0265	0.0001	
18-HETE	0.137±0.0175^{B}	0.182±0.0216	0.102±0.0154^{B}	0.173±0.028	0.268±0.036^{A}	0.456±0.105	0.258±0.0207^{A}	0.479±0.089	<.0001	0.0002
20-HETE	1.08±0.213^{A}	0.432±0.109	0.399±0.0731^{B}	0.289±0.0575	0.25±0.0533^{BC}	0.265±0.0733	0.196±0.0369^{C}	0.274±0.111	<.0001	
Sum	184±47.3^{A}	307±59.4	139±24.1^{A}	200±25.4	157±40.2^{A}	174±30.8	57.8±10.6^{B}	78.9±16.5	<.0001	0.012
LA derived oxylipins										
9-HODE	10.9±1.48	16.3±1.44	13.0±1.17	14.2±1.03	16.2±3.32	14.0±2.28	7.79±0.902	21.0±7.06		0.0104
9-oxoODE	0.368±0.0947^{bc}	0.287±0.0566^{c}	0.55±0.107^{ba}	0.375±0.0999^{bc}	0.869±0.193^{a}	0.587±0.0807^{ba}	0.272±0.0779^{c}	0.946±0.554^{ba}		0.0308
13-HODE	6.67±0.948^{bc}	11±1.33^{a}	8.05±0.469^{ba}	8.64±0.707^{ba}	10.4±1.85^{ba}	8.81±1.39^{ba}	5.15±0.533^{c}	12±3.39^{a}		0.0322
13-oxoODE	2.77±0.379^{bc}	2.19±0.337^{cd}	6.76±0.821^{a}	3.38±0.641^{bc}	4.24±0.742^{ba}	3.68±1.15^{bc}	1.59±0.48^{d}	3.46±1.79^{bcd}		0.0374
9,12,13- TriHOME	3.02±0.518^{dc}	4.42±0.744^{bdac}	3.31±0.343^{bdc}	5.01±0.898^{bac}	5.95±1.61^{ba}	3.43±0.468^{bdc}	2.76±0.256^{d}	7.26±2.74^{a}		0.0265
9,10-EpOME	0.0979±0.0273^{ba}	0.101±0.0276^{ba}	0.175±0.0254^{a}	0.0305±0.00805^{b}	0.122±0.041^{ba}	0.141±0.0513^{a}	0.0327±0.0127^{b}	0.177±0.0635^{a}		0.0039
9,10-DiHOME	1.63±0.3	1.58±0.257	1.4±0.0975	1.16±0.0982	1.55±0.264	1.29±0.236	0.888±0.0733	1.36±0.269		
12,13-EpOME	0.351±0.064^{ba}	0.348±0.0677^{ba}	0.442±0.0542^{a}	0.174±0.012^{c}	0.517±0.0923^{a}	0.389±0.1^{ba}	0.232±0.0133^{bc}	0.592±0.173^{a}		0.0021
12,13-DiHOME	1.24±0.227	1.61±0.392	2.19±0.241	1.39±0.157	1.95±0.447	1.36±0.484	0.739±0.0891	1.50±0.481		
Sum	27.0±3.41^{bc}	37.7±4.11^{ab}	35.9±2.18^{ab}	34.3±3.18^{abc}	41.8±7.63^{ab}	33.0±5.27^{abc}	19.5±2.22^{c}	46.4±11.3^{a}		0.0183
GLA derived oxylipins										
13-HOTrE-γ	0.293±0.0694^{A}	0.304±0.0767	0.221±0.0364^{A}	0.177±0.0185	0.149±0.0457^{B}	0.111±0.0347	0.0912±0.00872^{B}	0.152±0.0438	0.0004	
DGLA derived oxylipins										
PGD_1	0.174±0.0617^{A}	0.344±0.083	0.132±0.0128^{A}	0.282±0.0566	0.115±0.0546^{B}	0.151±0.0298	0.0593±0.0137^{B}	0.113±0.0437	0.0014	0.0005
$PGF_{1\alpha}$	0.0494±0.0227	0.102±0.0324	0.0319±0.021	0.123±0.0324	0.0317±0.0148	0.0283±0.0137	0.00862±0.00862	0.0474±0.0271		0.0091
8-HETrE	0.039±0.00401	0.0938±0.017	0.0507±0.00752	0.101±0.00864	0.0704±0.0197	0.0842±0.0126	0.0446±0.00415	0.104±0.0322		<.0001
15-HETrE	0.375±0.0588	1.04±0.167	0.426±0.0835	1.07±0.124	0.475±0.14	0.589±0.102	0.393±0.0776	0.692±0.142		<.0001
Sum	0.637±0.128	1.58±0.273	0.64±0.0875	1.57±0.209	0.691±0.221	0.852±0.147	0.506±0.0916	0.956±0.208		<.0001
Sum n-6	212±47.3^{A}	347±60.6	176±25.6^{A}	237±25.5	200±46.3^{A}	208±30.4	77.9±12.1^{B}	126±24.8	<.0001	0.0053
Sum n-3	15.2±1.01^{D}	21.9±2.9	88.2±15.4^{C}	106±11.1	372±105^{A}	375±56.1	129±13.8^{B}	289±89	<.0001	0.0095

n6/n3 Ratio	13.5±2.34[A]	16.0±2.32	2.09±0.184[B]	2.26±0.191	0.653±0.136[C]	0.59±0.074	0.601±0.051[C]	0.487±0.044	<.0001

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

# Denotes that oxylipin is not detected.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet × sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

*Denotes that since there is no primary standard, response factors cannot be determined and data for these oxylipins is not quantitative. These values are not included in the sums. AA oxylipin sum includes AA non-enzymatic products.

Values are mean±SE (n=5-6 for each), and are based on ng/ml serum..

Table 4.9 Serum Total Fatty Acid In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

Diet	Control		ALA		EPA		DHA		Effect	
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Diet**	**Sex**
					ug/ml					
C140	26.8±1.94	32.5±7.19	17.8±2.62	26.8±3.28	29.1±5.79	31.5±6.47	31.7±5.38	39.8±1.85		
C160	826±107^{A}	753±59.3	293±34.6^{B}	399±53.5	244±50.1^{B}	345±28.2	414±69.4^{B}	383±71.6	<.0001	
C161	78.3±12.5^{A}	103±17.4	37±14.2^{B}	36.1±7.93	14±4.07^{B}	29.1±4.09	20±5.98^{B}	33.1±9.08	<.0001	
C170	5.37±1.15^{ba}	3.22±0.938^{bc}	6.5±0.411^{a}	2.79±1.13^{c}	5.85±0.382^{ba}	0.529±0.198^{d}	4.88±1.64^{bac}	0.404±0.12^{d}		0.0138❖
C171	2.61±1.65	1.53±1.53	1.1±1.1	–	–	–	–	–		
C180	640±77.1^{A}	345±39.7	399±76.9^{B}	221±27.2	225±33.6^{C}	146±17.4	351±48.2^{C}	133±17	<.0001	<.0001
C181	1293±192^{A}	976±146	223±28.6^{B}	268±38.4	142±28.6^{C}	175±24.5	250±49.2^{BC}	202±48.6	<.0001	
C182n6	548±54.6^{A}	645±45.5	332±22.8^{C}	389±35.1	255±48.7^{D}	302±36.9	455±34^{B}	454±25	<.0001	
C183n3	27.1±3.2^{B}	38.7±5.12	165±19.3^{A}	215±22.8	19±4.3^{C}	20.8±4.23	25.5±3.13^{B}	26.9±1.99	<.0001	
C183n6	8.42±1.24^{A}	7.57±1.01	4.13±1.01^{B}	2.85±0.585	1.49±0.276^{C}	1.36±0.302	2±0.266^{C}	1.14±0.31	<.0001	
C200	4.85±0.335^{A}	5.86±0.513	2.02±0.299^{B}	2.52±0.27	2.52±0.387^{B}	2.36±0.22	3.11±0.237^{B}	2.46±0.0929	<.0001	
C201	7.58±1.27^{A}	9.37±1.57	0.722±0.167^{B}	0.722±0.0803	0.608±0.244^{BC}	0.416±0.13	0.478±0.103^{B}	0.519±0.169	<.0001	
C202n6	4.09±0.262^{A}	7.54±0.443	2.9±0.75^{B}	3.09±0.405	1.24±0.175^{C}	2.16±0.154	1.93±0.271^{C}	2.01±0.452	<.0001	0.0019
C203n3	0.479±0.0956^{B}	0.869±0.287	5.18±1.83^{A}	4.37±0.582	0.747±0.573^{B}	0.881±0.274	0.282±0.064^{B}	0.471±0.157	<.0001	
C203n6	16±1.44^{b}	27.4±4.65^{a}	14.6±2.9^{bc}	19.9±2.67^{ba}	6.1±0.723^{d}	8.88±1.04^{d}	14.0±1.85^{b}	9.21±1.46^{dc}		0.0131
C204n6	884±117^{a}	605±68.3^{b}	278±30.5^{c}	184±22.6^{d}	129±18.9^{ed}	135±12.0^{ed}	123±19.6^{e}	50.0±3.98^{f}		0.0076
C205n3	11.0±1.55^{e}	10.5±1.81^{e}	174±29.2^{c}	92.1±16.5^{d}	368±76.2^{ba}	396±47.3^{a}	242±57.8^{bc}	82.1±16.3^{d}		0.0163
C220	6.41±0.317^{ba}	7.48±0.384^{a}	4.90±0.727^{c}	4.37±0.512^{dc}	4.04±0.688^{dc}	3.28±0.492^{d}	5.42±0.471^{bc}	2.99±0.344^{d}		0.0151
C221	0.91±0.058^{A}	0.769±0.198	0.654±0.0994^{B}	0.466±0.176	0.341±0.0619^{B}	0.0988±0.0988	0.545±0.18^{B}	0.0331±0.0331	0.0097	
C222n6	0.704±0.66	0.485±0.375	–	–	–	–	–	–		
C224n6	6.96±0.411^{b}	9.91±0.591^{a}	0.735±0.342^{c}	0.533±0.201^{c}	0.328±0.125^{c}	0.127±0.093^{c}	0.442±0.193^{c}	0.0497±0.0373^{c}		<.0001
C225n3	5.21±0.66^{C}	10.2±1.01	24.9±7.5^{B}	27.4±5.67	43.9±7.04^{A}	68.0±9.21	33.1±7.15^{B}	34.8±5.11	<.0001	
C225n6	20.5±2.17^{A}	18.1±2.78	3.09±0.647^{C}	1.34±0.551	2.45±0.368^{C}	0.474±0.301	6.64±1.02^{B}	6.43±0.497	<.0001	0.0086
C226n3	152±18.0^{B}	79.7±5.78	106±24.1^{B}	81.2±12.2	47.6±9.4^{C}	48.6±5.14	397±65.0^{A}	366±50.6	<.0001	
C240	12.1±1.73^{A}	10.7±0.604	11.1±0.851^{AB}	9.37±1.11	7.88±0.968^{B}	7.58±0.939	9.83±0.708^{BC}	6.74±0.549	0.0019	0.0255
C241	12.7±0.937^{A}	13.8±1.81	8.84±1.10^{B}	11.1±1.23	4.61±0.493^{C}	5.21±0.96	6.66±0.576^{C}	4.68±0.446	<.0001	
n6/n3 Ratio	7.35±0.515^{b}	9.5±0.581^{a}	1.33±0.104^{c}	1.43±0.046^{c}	0.856±0.043^{ed}	0.82±0.015^{e}	0.996±0.07^{d}	0.937±0.035^{ed}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters

within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a diet×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

Values are mean±SE (n=5-6 for each), and based on ng per ml of serum.

4.3.2 Effect Of Dietary ALA

A 3% difference in the level of ALA in the ALA (3.26 g ALA/100 g oil) compared to control (0.26g ALA/100 g oil) diet consistently resulted in higher ALA and EPA, in total and all individual ALA oxylipins and total and 67-100% of individual EPA oxylipins in the kidney, liver and serum (Figures 4.2a, 4.3a, 4.4a; Tables 4.4a, 4.6a, 4.8a). In contrast, higher dietary ALA resulted in lower DHA in kidney, higher DHA in liver, and had no effect on DHA levels in serum. Despite this, total and 10/12 DHA oxylipins in kidney and total and 7/13 DHA oxylipins in serum were higher in rats given ALA compared to control diets, while DHA oxylipins in the liver were not different.

With respect to n-6 fatty acid and their oxylipins, higher dietary ALA compared to the control diet resulted in lower levels of AA in kidney, liver and serum and lower total AA oxylipins in kidney and serum, as well as many individual AA oxylipins (26-70%) in kidney, liver and serum (Figures 4.2b, 4.3b, 4.4b; Tables 4.4b, 4.6b, 4.8b). In addition, several AA oxylipins (3-9%) that were metabolites (e.g. 5,15-DiHETE in serum, 11β-dhk-$PGF_{2\alpha}$ in kidney) of initial oxylipins formed were higher. In contrast to AA, while the levels of LA were higher in liver and kidney and lower in serum, total LA oxylipins in the kidney and 11-25% of individual LA oxylipins in the kidney, liver and serum were higher in the ALA compared to control diet. Other n-6 fatty acid and their oxylipins were either lower or unchanged in tissues from rats given ALA compared to control diets.

4.3.3 Effect Of Dietary EPA

Higher dietary EPA in rats given the EPA (3 g EPA/100 g oil) compared to control (no EPA) diet resulted in higher EPA and all EPA oxylipins in all 3 tissues (Figures 4.2a, 4.3a, 4.4a; Tables 4.4a, 4.6a, 4.8a). In contrast, dietary EPA resulted in lower levels of

DHA in all 3 tissues (Figures 4.2, 4.3, 4.4; Tables 4.5,4.7,4.9), but this was not reflected in oxylipin levels, as 2/12 DHA oxylipins were lower in kidney, none were different in liver and 4/13 were higher in serum, in EPA compared to control rats. ALA levels were higher in kidneys and livers of EPA compared to control rats, but lower in serum. In comparison, 2/5 ALA oxylipins in kidney were lower in kidney, 1/3 was higher in liver, and none were different in serum.

With respect to effects on n-6 fatty acid, dietary EPA resulted in lower levels of AA and AdA in all tissues, DGLA in kidney and LA and GLA in serum, but resulted in higher levels of LA in kidney and liver. These effects were generally reflected in the differences in oxylipins derived from these n-6 fatty acid, including total and individual (30/38 in kidney and 22/32 in liver) AA oxylipins being lower in kidney and liver and 20/32 individual AA oxylipins being lower in serum. Similar to the effect of dietary ALA, providing dietary EPA resulted in some LA oxylipins (2/9) being higher in liver and serum; the latter despite having lower LA levels.

4.3.4 Effect Of Dietary DHA

A 3% difference in the level of dietary DHA in the DHA (3 g DHA/100 g oil) compared to control (no DHA) rats consistently resulted in higher DHA and EPA (Figures 4.2, 4.3, 4.4; Tables 4.5,4.7,4.9) and in total and all individual oxylipins derived from these fatty acid in all 3 tissues analyzed (Figures 4.2a, 4.3a, 4.4a; Tables 4.4a, 4.6a, 4.8a). In contrast, dietary DHA compared to the control diet resulted in more ALA only in kidneys and male livers and higher ALA oxylipins (total and 2/3 individual) only in liver.

With respect to effects on n-6 fatty acid, dietary DHA compared to the control diet resulted in lower levels of AA and AdA in all tissues, and DGLA (females only), LA and GLA in serum, but in higher levels of LA in kidney and liver. These effects were mostly

reflected in the differences in oxylipins derived from these n-6 fatty acid (including higher liver LA oxylipins – total and 5/9 individual), except that kidney and serum LA oxylipins were not different despite the higher kidney and lower LA levels in kidney and serum, respectively, in DHA rats. In addition, DGLA oxylipin levels were lower in kidney and liver, despite similar DGLA levels in tissues from rats given the DHA and control diets.

4.3.5 Comparison Of Dietary ALA, EPA And DHA Effects

Overall, dietary DHA resulted in the lowest ratio of oxylipins derived from n-6 compared to n-3 fatty acid, followed in order by the EPA, ALA and control diet for kidney, liver and serum (Figures 4.2b, 4.3b, 4.4b; Tables 4.4b, 4.6b, 4.8b).

When comparing the effects of different dietary n-3 fatty acid on n-3 fatty acid oxylipins, their effects were generally consistent in the kidney, liver and serum. Providing dietary ALA compared to EPA or DHA resulted in higher levels of total and all individual ALA oxylipins in kidney, liver and serum. Dietary ALA compared to EPA also resulted in more total and 11/12 individual DHA oxylipins in kidney and 3/13 individual DHA oxylipins in serum. Dietary EPA compared to ALA resulted in higher total and 88-100% of individual EPA oxylipins in kidney, liver and serum, and compared to DHA resulted in higher total EPA oxylipins in kidney and serum and 78-88% of individual oxylipins in kidney, liver and serum. Dietary DHA compared to ALA or EPA resulted in higher total and all individual DHA oxylipins in kidney, liver and serum, and compared to ALA it resulted in higher total EPA oxylipins in kidney and 25-67% of individual EPA oxylipins in all 3 tissues. DHA compared to EPA also resulted in higher total and 1/3 individual ALA oxylipins in liver.

When comparing the effects dietary n-3 fatty acid on n-6 fatty acid oxylipins, however, their effects were different in kidney and serum compared to liver (Figures 4.2,

4.3, 4.4; Tables 4.4,4.6,4.8). In kidney and serum, dietary EPA compared to ALA resulted in lower total and 22/38 (kidney) and 10/33 (serum) individual AA oxylipins, 3/8 LA oxylipins in kidney and 25-100% of DGLA and GLA oxylipins in kidney and serum. In the liver, on the other hand, while dietary EPA compared to ALA also resulted in lower total AA oxylipins, fewer (5/32) individual AA oxylipins were lower than in kidney and serum, and 5/9 LA oxylipins, 1/4 DGLA oxylipins and 1/1 EDA oxylipins were higher in liver. Similar tissue differences were also observed in n-6 fatty acid oxylipin effects when comparing dietary DHA to EPA or ALA. DHA compared to EPA resulted in lower levels of fewer AA oxylipins in liver (8/32) compared to kidney (17/38) and serum (23/33), and resulted in 1/9 LA, 1/4 DGLA and 1/1 AdA oxylipins being higher in the livers of DHA compared to EPA fed rats, while 5/9 LA oxylipins were lower in serum and 1/3 AdA oxylipins were lower in kidney. DHA compared to ALA also resulted in lower levels of total and (27/38) and (25/33) individual AA oxylipins, 1/8 and 5/9 LA oxylipins, 4/4 and 1/4 DGLA oxylipins in kidney and serum, respectively, as well as total LA oxylipins in serum and 2/3 AdA oxylipins in kidney. In the liver, on the other hand, while dietary DHA compared to ALA also resulted in lower total AA oxylipins, fewer (8/32) individual AA oxylipins were lower than in kidney and serum, and total and 6/9 LA oxylipins, 1/4 DGLA oxylipins and 1/2 AdA oxylipins were higher in liver.

In general, these differential effects of individual dietary n-3 fatty acid on oxylipins reflected their effects on fatty acid. The notable exception, however, was the effect on LA oxylipins in kidney and serum, which tended to be opposite the effect of LA in these two tissues (e.g. dietary DHA compared to ALA resulted in higher LA, but lower LA oxylipins in kidney and serum).

4.3.6 Sex Effects

Sex differences were observed in 20/83 oxylipins in kidney, 18/74 in liver and 40/74 in serum (Figures 4.2, 4.3, 4.4; Tables 4.4,4.6,4.8). Out of those with a sex effect, almost all were higher in males, with the following exceptions that were higher in females: $PGF_{3\alpha}$ in control fed rats, Δ^{17}-6k-$PGF_{1\alpha}$, and all DHA oxylipins in kidney; $PGF_{2\alpha}$, 16-HETE and 9,12,13-TriHOME in liver; $PGF_{3\alpha}$ and 17-HDoHE in serum. These sex effects on oxylipins did not necessarily coincide with fatty acid levels. For example, kidney LA is higher in males and ALA is higher in females, but oxylipins from these fatty acid were either higher in males or did not have a sex effect (Tables 4.5,4.7,4.9).

To further investigate sex effects on oxylipin formation, the ratios of oxylipins to parent phospholipid fatty acid (in kidney and liver) and total fatty acid (in serum) were determined for oxylipins formed from multiple fatty acid via the following pathways: COX/PGE synthase, 5-, 12-, and 15-LOX, CYP hydroxylase (fatty acid hydroxylated at n-2 selected), and CYP epoxygenase. These analyses revealed that oxylipin formation in males was greater than or equal to females for all oxylipins for all fatty acid substrates (Table 4. 10. a-f). This was even the case for DHA derived oxylipins in kidney that were higher in females. An example of the product/substrate ratio for each enzyme in rats fed control diet is shown in Figure 4. 5. a-f; data for all ratios in all diet groups and tissues are shown in Table 4. 10. a-f.

4.3.7 Effect Of Fatty Acid Type And Chain Length On Oxylipin Formation

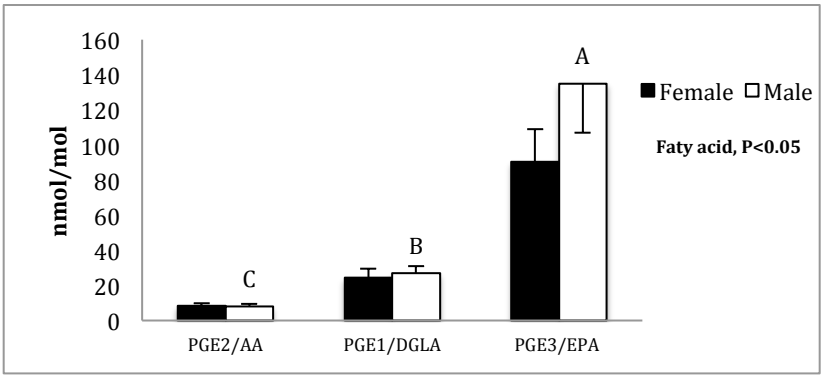
Product to fatty acid ratios (Table 4.10. a-f) also were used to examine the relative rates of oxylipin formation from different fatty acid. These ratios demonstrate that oxylipin formation is higher from n-3 compared to n-6 fatty acid. In addition, the order of

fatty acid conversion to oxylipins was 18-carbon ≥ 20-carbon ≥ 22-carbon in most, but not all cases. This pattern was consistent in almost all (22/24) such comparisons in liver, 15/24 in kidney and 16/24 in serum. The exceptions in these comparisons were most often due to DHA compared to EPA comparisons (e.g. in kidney ALA and DHA groups, where 14-HDoHE/DHA was higher than 12-HEPE/EPA) or due to DGLA compared to LA comparisons (e.g. in serum EPA group, where 15-HETrE/DGLA was higher than 13-HODE/LA).

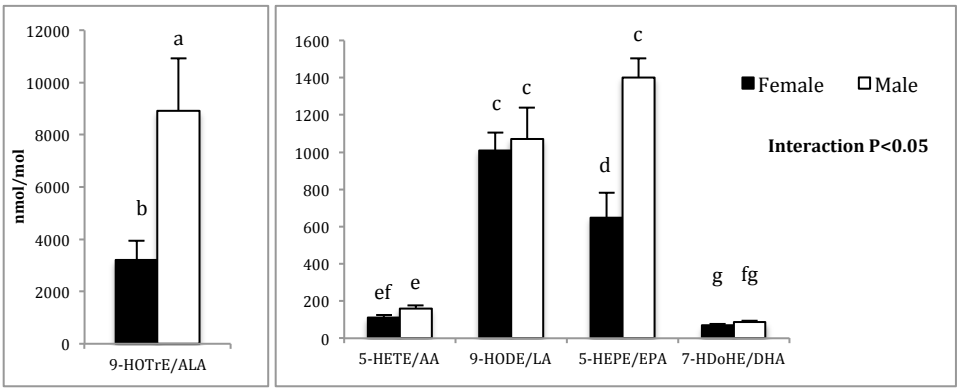
Figure 4.5 a-f. Example Of Product/Precursor Ratio Results Illustrating Patterns Found In All Tissues And Diets.

Shown are the product/precursor ratios for each enzyme in kidney of rats provided control diets for 6 weeks. Ratios with differing letters are significantly different. For comparisons for all tissues and diets, see Table 4.10 a-f.

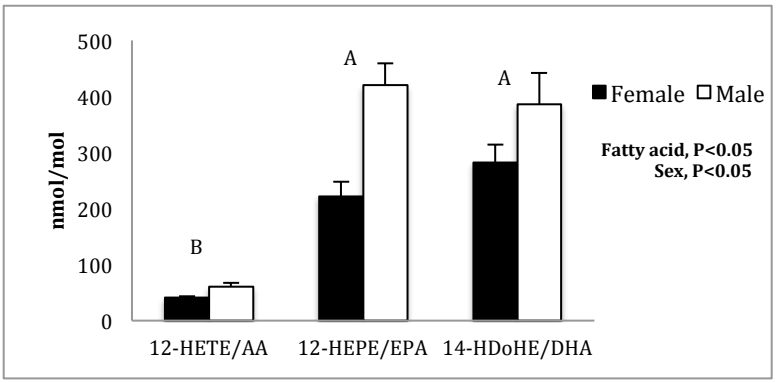
5a. COX-PGE synthase



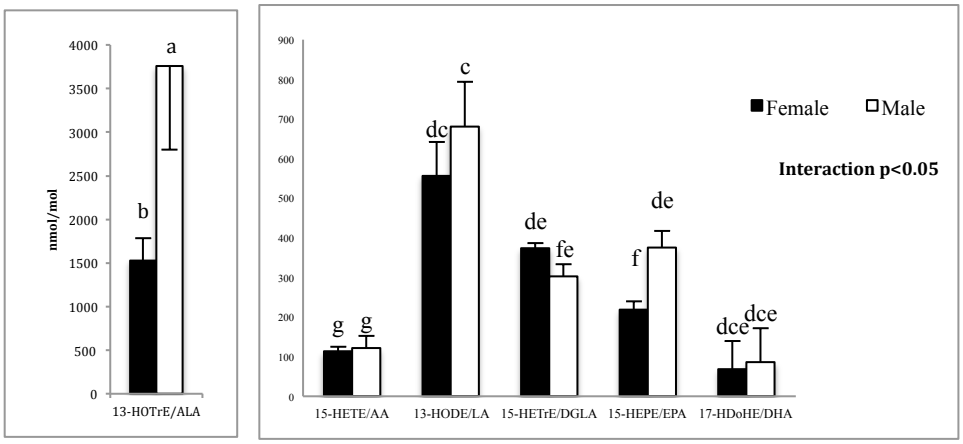
5b. 5-LOX



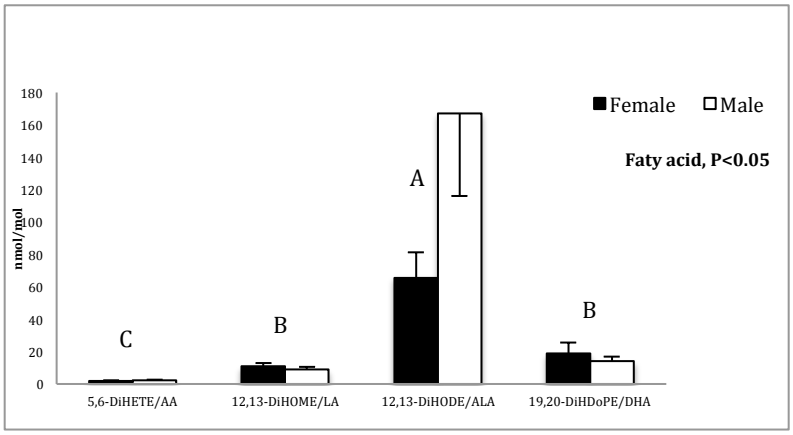
5c. 12-LOX



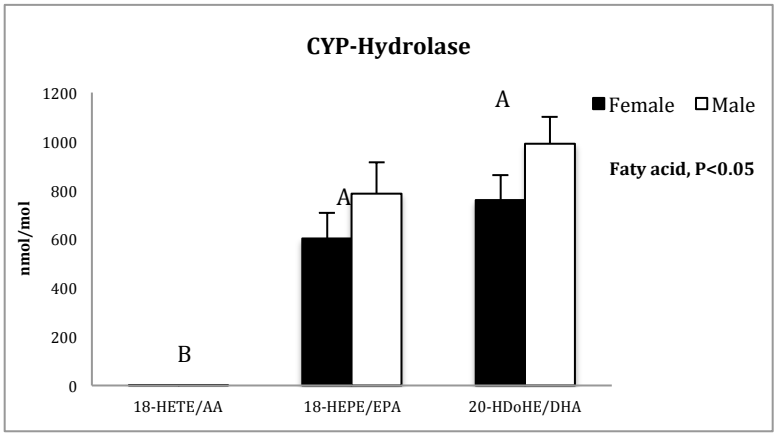
5d. 15-LOX



5e. CYP-Epoxygenase



5f. CYP-Hydrolase



Differing upper case superscript letters shown on the female values indicated differences between diets.
Differing lower case superscript letters differences between means.
n=5-6 for each.

Table 410. Product/Precursor Ratios In Rats Given Control, DHA, EPA, ALA Diets For Six Weeks.

a. COX-PGE synthase

		PGE_2/AA		PGE_1/DGLA		PGE_3/EPA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Control	8.37±1.46^{C}	8.12±1.41	24.3±5.35^{B}	27.0±4.11	90.3±18.7^{A}	135±28	<.0001	
	ALA	10.0±1.67^{C}	11.2±1.55	31.8±9.04^{B}	24.9±4.23	78.2±14.6^{A}	86.5±17.1	<.0001	
	EPA	7.6±1.61^{B}	8.73±1.04	14.8±4.53^{B}	14.6±2.05	81.8±17.7^{A}	88.0±10.8	<.0001	
	DHA	3.9±1.02^{c}	5.86±0.48^{c}	6.65±1.25^{c}	7.3±0.93^{c}	293±154^{a}	39.1±5.57^{b}	0.0294❖	
Liver	Control	7.01±1.53^{c}	14.1±1.54^{c}			49.1±17.7^{b}	272±44.0^{a}	0.0271	
	ALA	3.23±0.770^{c}	4.44±0.57^{c}			34.2±10.5^{b}	66.7±9.90^{a}	<.0001	
	EPA	9.71±2.0^{B}	12.1±3.04			135±10.6^{A}	257±64.4	<.0001	
	DHA	7.84±2.32^{B}	10.3±1.16			76.6±20.4^{A}	150±22.0	<.0001	0.0103
Serum	Control	3.85±1.09	10.5±3.04						0.0491
	ALA	7.07±1.19^{A}	21.0±6.92			0.49−.16^{B}	1.76±0.530	<.0001	0.0005
	EPA	10.7±4.05^{A}	13.8±4.50			1.66±1.21^{B}	3.73±1.34	0.0001	0.0373
	DHA	5.78±2.02^{A}	17.8±7.52			0.42±0.17^{B}	2.78±0.810	<.0001	0.0004

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=4-6)

b. 5-LOX

		5-HETE/AA		9-HODE/LA		9-HOTrE/ALA		5-HEPE/EPA		7-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol										Fatty Acid	Sex
Kidney	Control	111±12.5^{ef}	161±17.4^{e}	1010±96.2^{c}	1070±168^{c}	3200±749^{b}	8910±2020^{a}	649±132^{d}	1400±105^{c}	68.6±9.52^{g}	86.5±6.68^{fg}	0.0086❖	
	ALA	155±15.9^{D}	188±21.7	956±147^{B}	1120±283	3020±313^{A}	5380±1680	488±35.3^{C}	777±139	141±20.4^{D}	186±34.5	<.0001	0.0264
	EPA	112±18.8^{C}	126±10.7	610±163^{B}	569±105	1500±513^{A}	2110±306	706±183^{B}	714±74.2	90.7±19.1^{C}	92.2±14.2	<.0001	
	DHA	98.5±8.77^{D}	109±8.72	540±53.3^{B}	611±57.9	1650±228^{A}	3070±816	234±26.2^{C}	345±59.5	228±21.9^{C}	311±61.4	<.0001	0.005
Liver	Control	1.07±0.44^{f}	2.07±0.47^{e}	751±164^{b}	980±176^{b}	7860±2250^{a}	10400±2400^{a}	188±45.9^{c}	568±195^{b}	19.8±4.01^{d}	31.4±3.32^{d}	<.0001	
	ALA	25.6±6.95^{ed}	53.8±9.51^{cd}	344±125^{b}	380±128^{b}	7520±2740^{a}	12700±5590^{a}	67.1±17.1^{cd}	142±24.2^{cb}	16.2±4.07^{e}	36.2±21.8E^{d}	<.0001	
	EPA	59.8±9.76^{C}	89.9±22.1	1240±192^{B}	957±342	10000±3320^{A}	5230±939	480±65.2^{B}	1280±453	32.9±6.75^{D}	54.3±25.3	<.0001	
	DHA	80.4±23.5^{D}	101±22.5	907±218^{B}	823±193	16500±3470^{A}	16600±5380	210±53.9^{C}	482±88.1	125±54.5^{D}	176±68.6	<.0001	
Serum	Control	2.41±0.32^{d}	5.55±0.55^{c}	25.6±6.92^{a}	24.1±1.8^{a}	18.8±3.58^{ba}	21.9±4.11^{a}	4.97±0.44^{c}	13.7±2.17^{b}	1.33±0.140^{e}	1.87±0.260^{ed}	0.0065	
	ALA	4.21±0.47^{c}	11±1.38^{b}	33.1±5.41^{a}	37.1±6.01^{a}	40.7±10.4^{a}	38.9±5.3^{a}	3.95±0.63^{bc}	12.1±2.18^{b}	2.22±0.30^{d}	3.56±0.40^{c}	0.0083	
	EPA	9.36±2.61^{B}	11.2±1.31	84.7±31.9^{A}	50.6±13.3	76±36^{A}	56.3±25.2	19.6±8.51^{B}	27.5±9.44	5.49±1.38^{C}	4.42±0.540	<.0001	
	DHA	4.15±0.74^{f}	22.5±6.10^{bac}	16.4±1.87^{bdc}	84.4±55^{a}	18.8±1.99^{bc}	35.6±10.5^{ba}	5.44±1.2^{ef}	43.4±24.1^{ba}	9.71±4.03^{edf}	18.7±11.4^{edc}	0.0002	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed.

Values are mean±SE (n=4-6)

c. 12-LOX

		12-HETE/AA		12-HEPE/EPA		14-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Control	41.2±2.25^{B}	61±6.67	222±26.3^{A}	420±38.7	282±31.7^{A}	386±56.4	<.0001	0.0001
	ALA	80.5±9.95^{C}	108±22.9	261±33.5^{B}	392±96.8	558±71.6^{A}	921±207	<.0001	0.0376
	EPA	51.3±14.2^{B}	59.6±6.47	264±61.4^{A}	266±19.5	353±84.8^{A}	366±53.9	<.0001	
	DHA	44.3±6.88^{C}	50.6±6.56	130±18.2^{B}	182±43.3	778±92^{A}	1140±267	<.0001	
Liver	Control	168±36.5^{B}	187±44.3	927±85.2^{A}	920±209	148±35.6^{B}	184±32.5	<.0001	
	ALA	151±84.7^{B}	108±31.6	600±316^{A}	419±88.4	188±89.9^{B}	236±135	0.0217	
	EPA	85.5±19.5^{C}	62.5±9.82	842±193^{A}	834±138	186±48.5^{B}	200±56.3	<.0001	
	DHA	332±124^{B}	227±50.8	1010±320^{A}	1080±252	941±273^{A}	959±287	<.0001	
Serum	Control	140±34.9^{A}	279±41.9	194±47.6^{A}	523±153	27.9±6.63^{B}	63±12.9	<.0001	0.0002
	ALA	347±69^{A}	765±211	263±39.7^{A}	730±217	57.6±11.2^{B}	101±18.0	<.0001	0.0003
	EPA	1030±293^{A}	974±192	1210±544^{A}	1290±538	190±60^{B}	148±20.7	<.0001	
	DHA	404±90.2^{A}	1140±232	208±50.8^{A}	1050±472	106±41.6^{B}	298±206	<.0001	0.0006

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets.
Values are mean±SE (n=4-6)

d. 15-LOX

		15-HETE/AA		13-HODE/LA		15-HETrE/DGLA		13-HOTrE/ALA		15-HEPE/EPA		17-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol												Fatty Acid	Sex
Kidney	Control	114±10.7^{g}	122±30.7^{g}	557±85.1^{dc}	681±113^{c}	374±13.2^{de}	303±29.7^{fe}	1530±257^{b}	3760±960^{a}	219±20.2^{f}	376±41.5^{de}	453±71.3^{dce}	490±86.1^{dce}	0.0331❖	
	ALA	141±23.4^{D}	238±41.8	608±76.5^{C}	687±165	406±34.9^{C}	461±92	1700±144^{A}	2990±862	167±29.2^{D}	304±60.6	967±146^{B}	1190±296	<.0001	0.0283
	EPA	90.2±21.8^{D}	112±26.5	432±103^{B}	384±68.3	208±38.3^{C}	203±20.6	972±285^{A}	1490±316	201±75.5^{C}	203±35.5	717±204^{B}	630±153	<.0001	
	DHA	125±10.9^{D}	134±8.75	285±24.1^{B}	340±32.8	203±35.8^{C}	209±32.9	1230±134^{A}	2120±560	102±13.5^{D}	145±40.9	1690±152^{A}	2050±358	<.0001	0.0422
Liver	Control	79.1±19.1^{h}	189±38.3^{gf}	332±85.2^{ef}	463±91.6^{ed}	971±207^{abc}	656±174^{bcd}	1050±170^{ab}	1810±412^{a}	549±28.9^{bcd}	544±104^{cde}	104±16.6^{gh}	200±25.6^{f}	0.0465	
	ALA	69.6±20.7^{f}	64.4±14.5^{f}	148±47.0^{fe}	182±78.1^{fecd}	471±211^{bc}	285±81.8^{bcd}	816±175^{ba}	1530±653^{a}	293±145^{fecd}	253±38.8^{becd}	134±37.4^{fed}	224±123^{fecd}	<.0001	
	EPA	146±23.9^{C}	143±45.2	497±93.5^{B}	424±159	261±73.3^{BC}	294±84.1	1340±382^{A}	1550±673	375±80.9^{B}	499±69	288±65^{B}	395±166	<.0001	
	DHA	203±48.5^{C}	179±43.2	476±139^{B}	347±79.3	794±262^{B}	431±126	2510±666^{A}	3750±1700	602±273^{B}	620±140	753±277^{B}	811±376	<.0001	
Serum	Control	9.11±2.24^{d}	23.4±3.74^{bc}	15.6±4.02^{c}	16.2±1.66^{c}	0.0235±4.53^{bc}	42.6±12^{ba}	35.2±10.0^{b}	33.8±7.26^{b}	5.69±0.82^{d}	14.9±1.81^{c}	22.4±3.02^{bc}	58.9±7.05^{a}	0.0253	
	ALA	18.2±2.79^{f}	53.5±15.2^{bc}	20.6±2.92^{fe}	22±2.78^{fe}	31.4±6.04^{de}	56.2±11.4^{bc}	67.9±11.3^{ab}	52±4.03^{bc}	5.61±0.61^{g}	19.8±2.61^{fe}	35±4.68^{cd}	91.4±11.1^{a}	<.0001	
	EPA	32.7±10.5^{BC}	38.5±9.27	54.4±19.2^{BC}	32.1±8.73	81.9±25.6^{A}	65.6±12.0	104±48.4^{AB}	63.7±24.4	21.3±9.03^{C}	26.8±8.18	92.9±20.4^{A}	90.1±12.9	<.0001	
	DHA	15.3±4.24^{egf}	55.4±10.1^{bdc}	10.9±1.26^{gf}	45.9±27.8^{edc}	28.7±6.3^{edc}	84.6±24.7^{ba}	23.5±2.99^{edf}	0.125±0.0893^{bac}	6.35±1.33^{g}	32.1±13.1^{edc}	90.3±41.1^{ba}	175±87.4^{a}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=4-6)

e. CYP-Epoxygenase

		5,6-DiHETE/AA		12,13-DiHOME/LA		12,13-DiHODE/ALA		19,20-DiHDoPE/DHA		Effect	
		Female	Male	Female	Male	Female	Male	Female	Male		
Tissue	Diet	Unit: nmol/mol								Fatty Acid	Sex
Kidney	Control	1.8−.320^{C}	2.28±0.50	10.8±2.23^{B}	8.87±1.78	65.5±16.0^{A}	167±50.9	18.8±6.67^{B}	14.2±2.64	<.0001	
	ALA	3.37±0.72^{D}	2.55±0.34	8.39±1.22^{C}	6.86±1.18	78.7±10.6^{A}	112±25.7	19.1±3.15^{B}	19.3±3.12	<.0001	
	EPA	1.79±0.40^{D}	2.48±0.39	7.55±1.79^{C}	4.91±0.59	48.4±16.6^{A}	44.7±15.3	11.9±2.78^{B}	11.9±2.26	<.0001	
	DHA	1.79±0.48^{D}	1.68±0.28	5.06±1.03^{C}	3.68±0.77	34.4±11.0^{A}	52.5±10.9	22.4±2.29^{B}	22.8±5.38	<.0001	
Liver	Control	3.74±1.4^{C}	12.2±2.79	32.0±8.08^{A}	121±23.8			12.2±2.75^{B}	68.8±11.2	<.0001	<.0001
	ALA	1.58±0.53^{B}	6.00±1.44	16.2±2.43^{A}	42.2±9.22			11.6±1.31^{A}	46.9±12.4	<.0001	<.0001
	EPA	2.31±0.190^{B}	7.5±1.63	23.2±2.96^{A}	73.3±25.4			15±3.21^{A}	69.9±23.3	<.0001	<.0001
	DHA	3.28±0.81^{C}	8.56±1.75	19.8±2.27^{B}	33.8±6.16			40.1±7.51^{A}	120±27.0	<.0001	<.0001
Serum	Control	0.43±0.080^{e}	1.78±0.37^{d}	2.44±0.410^{bcd}	2.26±0.58^{cd}	4.24±1.22^{a}	3.09±0.570^{cd}	3.29±0.560^{bc}	5.68±1.29^{a}	0.0006❖	
	ALA	1.02±0.250^{c}	3.77±0.66^{b}	5.19±0.820^{b}	3.33±0.46^{b}	4.84±1.05^{b}	4.22±0.50^{b}	6.25±1.02^{b}	15.6±5.09^{a}	0.0005	
	EPA	1.81±0.480^{c}	3.19±0.370^{bc}	9.43±3.95^{ba}	5.12±2.51^{bc}	16.5±8.82^{a}	9.59±5.49^{ba}	8.52±1.49^{ba}	10.9±1.18^{a}	0.0005	
	DHA	0.84−.100^{d}	4.57±0.580^{b}	1.46±0.17^{dc}	5.55±3.57^{bc}	4.21±1.06^{b}	12.3±9.33^{ba}	12.9±4.42^{a}	18.2±8.38^{a}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=4-6)

f. CYP-Hydrolase

		1 /AA		18-HEPE/EPA		20-HDoHE/DHA			
		Female	Male	Female	Male	Female	Male	Effect	
Tissue	Diet	nmol/mol						Fatty Acid	Sex
Kidney	Control	0.52±0.10^{B}	0.41±0.08	603±104^{A}	787±127	760±102^{A}	990±110	<.0001	
	ALA	0.36±0.05^{C}	0.96±0.22	9.40±1.31^{B}	20.9±3.15	9.90±2.09^{A}	13.4±1.73	<.0001	
	EPA	0.9−.21^{C}	1.01±0.24	560±200^{B}	451±105	846±219^{A}	787±102	<.0001	
	DHA	1.24±0.10^{C}	1.29±0.13	290±26.9^{B}	292±40.6	1630±150^{A}	1800±415	<.0001	
Liver	Control	1.01±0.23^{B}	2.95±0.74	161±35.4^{A}	408±149	104±28.4^{A}	251±47.9	<.0001	0.0003
	ALA	0.88±0.27^{B}	2.13±0.21	76.9±20.6^{A}	88.8±15.9	101±27.7^{A}	193±94.1	<.0001	<.0001
	EPA	8.73±2.76^{c}	29.4±12.2^{b}	427±61.3^{a}	753±257^{a}	264±54.9^{a}	411±146^{a}	<.0001	
	DHA	3.80±1.50^{c}	15.1±1.6^{b}	247±73.0^{a}	421±132^{a}	612±172^{a}	684±186^{a}	0.0299❖	
Serum	Control	0.16−.02^{C}	0.29−.04	5.42±0.56^{A}	11.0±1.89	3.13±0.29^{B}	6.79±1.31	<.0001	<.0001
	ALA	0.87−.15^{B}	0.76−.09	460±63.0^{A}	728±161	1600±270^{A}	1730±169	<.0001	<.0001
	EPA	2.2−.43^{C}	3.21±0.68	34.6±13.9^{A}	55.7±21.6	18.0±5.14^{B}	17.7±2.38	<.0001	
	DHA	2.33±0.47^{c}	8.96±1.25^{b}	9.17±2.02^{b}	60.1±27.7^{a}	36.3±19.3^{a}	76.0±43.5^{a}	<.0001	

Differing upper case superscript letters shown on the female values with in a row indicate differences between diets. Differing lower case superscript letters within a row indicate differences between means.

❖Denotes that the P values listed across diet and sex effects are the P value for a fatty acid×sex interaction when there was a significant interaction or the P value for the Wilcoxin test if the data were not normally distributed. Values are mean±SE (n=4-6)

4.4 Discussion

4.4.1 ALA Increases DHA Oxylipins Without Increasing DHA Levels

The rate of ALA conversion to DHA is very low. For example, using [U-^{13}C] ALA in non-human primates, the conversion from ALA to DHA was calculated to be 0.23% (41) and 0.57% (2) in two studies. In humans the conversion is also poor and the evidence is controversial and less consistent. For example, the extent of conversion from ALA to DHA is 4% (4), but some other research failed to detect significant DHA synthesis (42) or measured less than 0.05% of ALA being converted to DHA (3). Our laboratory has previously shown that ALA feeding can elevate renal DHA and EPA oxylipins in mice with kidney disease (11). However, whether this occurs in normal kidneys, or how the formation of oxylipins from ALA, DHA and EPA compares was not known. In the current study, with 3 g more of ALA in the ALA compared to the control diet, DHA was lower in kidney, not different in serum and higher in liver. In comparison, DHA oxylipins were higher in kidney and serum. Inconsistency of tissue fatty acid composition with the oxylipin profile also was observed in kidneys from obese rats given high fat diets (43). In our study most DHA oxylipins in kidney and serum were significantly increased, as well as the total DHA oxylipins (~60% higher in kidney in ALA group and ~50% higher in serum). This indicates that ALA conversion to DHA is able to increase DHA oxylipins even when the level of DHA is not increased. Most of the functions of the DHA oxylipins are beneficial, such as 14-HDoHE inhibiting platelet aggregation (9), 17-HDoHE having anti-inflammatory effects (7) and many other DHA oxylipins having beneficial health effects, as reviewed in (44).

The reason that liver has higher DHA may be due the fact that the liver is the major site for fatty acid conversion of ALA to DHA (2, 45, 46). However, even with a higher

DHA level, no DHA oxylipins in liver were increased. This discrepancy, along with all of the other data in Table 4. 3-9, further shows that fatty acid levels do not always determine oxylipin levels, and that in kidney and serum, ALA can increase DHA oxylipins.

4.4.2 DHA And EPA Effects On N-3 Oxylipins

For DHA and EPA feeding, the current study demonstrated that the n-3 oxylipin production is highly dependent on dietary intake. DHA or EPA feeding caused a pronounced increase in the n-3 oxylipins in kidney, liver and serum. This is the first study to show individual DHA or EPA specific effects on oxylipin production.

Dietary DHA increased all the DHA oxylipins in kidney, liver and serum from 300% to 2600%. Previous studies with DHA that showed an increase in DHA oxylipins used DHA in combination with EPA or other fatty acids (25, 47-49). Similar results were observed with high DHA combined with AA intake, which increased piglet heart and liver DHA and EPA levels. Also, with DHA plus AA intake, the level of both DHA and EPA oxylipins were increased (48), which suggests that retroconversion occurred. The retroconversion of fatty acids is also supported by a study in which a single dose of DHA increased the level of EPA in both rats and humans (13), and was confirmed in our study in rats. Our research showed that both EPA and EPA oxylipins were elevated after DHA intake, which demonstrates that retroconversion occurs sufficiently to affect oxylipin levels.

ALA and ALA oxylipins were also increased in liver after DHA intake. There are few studies on the effect of fish oil on ALA and its oxylipins (24, 25, 47, 48). Some studies with fish oil feeding examined fatty acid levels, but did not look into ALA oxylipins (49-53). Some studies in pigs and humans showed that with fish oil intervention, there were no alterations in ALA oxylipins (24, 48, 54). However other studies in humans (25, 26, 47) showed that fish oil feeding decreases ALA oxylipins in serum and plasma. However, except for one study, which provided a mixture of DHA and AA (48), all the

others provided an EPA/DHA mixture. For example, (25) used 1.56g EPA (~2%E) and 1.14g (~1.6%E) DHA daily; (47) used 1.1g (~1.6E%) EPA and 0.74g (~1E%) DHA daily; (26) used 4g (~6%E) EPA and 2g (~3%E) DHA daily. So the effects of DHA on ALA oxylipin production is unclear because all of the previous studies used a mixture of fish oil or a mixture of DHA in combination with other fatty acids.

Similar to DHA, dietary EPA increased EPA derived oxylipins in kidney, liver and serum (50%-13000%). EPA feeding also decreased DHA levels in kidney, serum and female livers. There are very few studies that have examined the effect of EPA on DHA; in one, it is reported that a single dose of EPA does not alter human plasma DHA levels (23), which is inconsistent with our findings of reduced DHA in rat tissues. Further, we observed discrepancies between DHA and DHA oxylipins in serum and liver, with serum displaying higher levels of DHA oxylipins and no change in liver DHA oxylipins despite lower DHA levels in these tissues. There is no previous research on the effect of dietary EPA alone on DHA oxylipins.

EPA feeding also increased ALA in kidney and liver but decreased ALA in serum. In comparison, 2 ALA kidney oxylipins were lower and 1 liver oxylipin was higher. There is little previous research on the effect of EPA alone on ALA derived oxylipins, and the few that did used a single dose of EPA and did not measure ALA (23, 55). One of the studies that measured ALA after a single dose of EPA feeding reported that ALA was not altered in rat liver (22), but another reported that ALA was lower after EPA intake in human serum (17). In the current study it appears that retroconversion from EPA to ALA fatty acid may occur in kidney and liver, but not in serum, but this was not necessarily reflected in the ALA oxylipins in the tissues examined.

4.4.3 ALA, DHA And EPA Effect On N-6 Oxylipins

In addition to effects on n-3 oxylipins, oxylipins from n-6 fatty acid such as AA, LA, DGLA and other n-6 fatty acid are also altered by n-3 fatty acid intake. Our data provided

evidence that DHA, EPA and ALA decreased AA and its oxylipins. AA derived oxylipins have various effects, such as PGD_2 inhibits human platelet aggregation (56), 5-HETE stimulates human cancer cell proliferation (57) and 5,6-EpETrE has vasodilatory effects (58). Oxylipins from n-6 fatty acid also play important roles in renal and liver health. For example, COX products are involved in vascular oxidative stress and endothelial dysfunction in renal interlobar arteries (59); and TXA_2 mediates microcirculatory failure in liver (60). Suppression of AA and AA derived oxylipins with combined DHA and EPA has been reported in human plasma, human serum, piglet plasma and mouse plasma (24-26, 47-50, 52-54, 61, 62), and some of these studies have shown that AA levels do not always correspond to oxylipin levels (26, 47, 48). However, all of those studies used a mixture of DHA and EPA.

For n-6 fatty acid other than AA, there are few studies that look at LA or its oxylipins. Some research has shown that a mixture of DHA and EPA decreases LA oxylipins in both human plasma and piglet plasma, along with either unchanged or decreased LA (24-26, 47, 48). LA fatty acid levels in serum were decreased by DHA, EPA and ALA in our study, which is similar to (25, 26). The LA derived oxylipins are the most abundant oxylipins detected in serum in our study and they are reported to have numerous physiological effects, including 13-HODE which inhibits platelet binding to endothelial cells (63), 9-HODE which inhibits proliferation and induces apoptosis (64). This suggests that alteration of LA oxylipins should not be over looked when evaluating the overall effects of dietary n-3 fatty acid. In our study, increasing DHA, EPA or ALA decreased serum LA, while increasing its levels in kidney and liver. Along with increased LA in these latter tissues, LA oxylipins also were higher in liver in all n-3 fatty acid diets and in ALA fed kidneys. In contrast, the decreased serum LA was associated with no change or higher levels of LA oxylipins. These unique effects may be due to the fact that only DHA or EPA were used in our study, while other studies have used mixtures of DHA and EPA. In addition to AA and LA and their oxylipins, DHA, EPA and ALA

feeding decreased many other n-6 oxylipins. For example, DGLA oxylipins are decreased in all three groups in kidney, liver and serum, as well as GLA oxylipins and most AdA oxylipins. While the AA oxylipins have been characterized as primarily pro-inflammatory, the properties of the other n-6 fatty acid oxylipins have not been as well characterized. For example, 13-HODE inhibits proliferation of hyper proliferative skin (65); 9,10 and 12,13-DiHOME cause mitochondrial dysfunction (66); 13-oxo-ODE reduces inflammation (67); PGE_1 vasodilates the rat coronary and systemic circulation (68); 12-HETrE inhibits human platelet aggregation (69). These will need to be characterized more fully to better understand the effects of dietary n-3 fatty acid on these oxylipins.

4.4.4 DHA And EPA Comparison

Currently around the world there are no specific dietary recommendations for EPA vs. DHA intake and the relative importance of these dietary fatty acid remains unclear (28, 70). DHA and EPA have different effects on lipoprotein and fatty acid metabolism: DHA increases HDL and EPA decreases HDL in human serum (17), EPA but not DHA lowers triacylglycerol in rat plasma and increases hepatic mitochondrial fatty acid oxidation in rat liver, which suggests that EPA is the more metabolically active fatty acid (71). Similarly, in contrast to DHA, EPA decreases availability of fatty acids for triacylglycerol synthesis by increasing mitochondrial β-oxidation in rat liver (72). EPA but not DHA, induces 2,4-dienoyl CoA reductase gene expression, which is an enzyme necessary for oxidation of unsaturated fatty acids (22). These suggest that, DHA differs from EPA with respect to lipid metabolism.

The specific effects of EPA and DHA on oxylipins, however, are not clear. Research on n-3 fatty acid and oxylipins have most often used a mix of EPA and DHA but did not compare them to each other (23-26, 47-50, 52-54, 61, 62). A recent study with individual EPA and DHA intake suggested that the different oxylipin profiles indicated that

postprandial changes in the n-3 oxylipins were likely to play a mechanistic as in vascular responses to EPA and DHA intake (23). In human pulmonary arteries, the long chain n-3 oxylipins account for ~75% of the capacity of DHA to elicit vasodilation (73). It is unknown how EPA and DHA have effects on oxylipin production including effects on other fatty acid derived oxylipins. This is important because a change in one type of oxylipins has effects on the whole oxylipin profile. In our study, DHA had a greater effect on lowering AA as well as AA oxylipins when compared to the EPA group, as AA derived individual oxylipins were ~10%-90% lower in the DHA group in kidney, liver and serum. Most AA derived oxylipins have pro-inflammatory effects (e.g. PGD_2) (74), platelet aggregation effects (PGE_2) (75) and stimulate cell proliferation (5,6-EpETrE) (76). This may be related to findings such as the one that showed that DHA improved postprandial arterial stiffness as assessed by augmentation index, but EPA did not (23).

In addition to altering AA derived oxylipins, DHA and EPA feeding also alter the DHA and EPA oxylipin profile. Although both DHA and EPA oxylipins are n-3 oxylipins, they have different functions. For example 17,18-EpETE from EPA is less vasoactive than EpDPE from DHA when comparing activation of BK channels (73, 77, 78). Our research provides data on the effect of DHA or EPA on the oxylipin profile, which may help differentiate the effects of EPA from DHA. However, more studies comparing the effects of these oxylipins need to be done in order to begin to address this issue.

4.4.5 Comparison Of Efficacy Of DHA, EPA And ALA.

The current study showed that DHA compared to ALA decreased the level of AA oxylipins by 75%-163% in the kidney, liver and serum. EPA compared to ALA also decreased the level of AA oxylipins by 128% and 154% in kidney and liver, respectively, but did not alter the ALA oxylipin levels in the serum. DHA compared to EPA decreased the level of AA oxylipins by 51% and 142% in kidney and serum, respectively, but not in

the liver. This generally suggests that DHA>EPA>ALA with respect to the ability of these n-3 fatty acid to reduce AA derived oxylipins. Further, the DHA compared to ALA groups had higher EPA oxylipin levels in kidney, liver and serum, which suggests that the retroconversion from DHA to EPA is greater than the conversion of ALA to EPA. These comparisons suggest that DHA and EPA are more effective in mediating DHA+EPA oxylipin changes than ALA. On the other hand, ALA compared to EPA feeding elevated more DHA oxylipins (11 in kidney and 3 in serum), suggesting that ALA is more effective than EPA in increasing DHA oxylipin levels. However, DHA itself was much more effective in elevating DHA oxylipins.

4.4.6 Sex Effect

The sex effect was similar to the results from the last chapter, which is that almost all the oxylipins with a sex effect were higher in males, with few exceptions (one being that all the DHA oxylipins with a sex effect are higher in females in kidney in both chapters). In addition, $PGF_{3\alpha}$ in kidney and serum, Δ^{17}-6k-$PGF_{1\alpha}$ in kidney were higher in females in both chapters. $PGF_{3\alpha}$ is higher in woman in early labour than in late labour (79), suggesting that there may be a function of $PGF_{3\alpha}$ on human pregnancy and parturition. But there is no information on sex effects on Δ^{17}-6k-$PGF_{1\alpha}$. There are also oxylipins that are different in these two chapters. For example, $PGF_{2\alpha}$, 16-HETE in liver are higher in females in this chapter but not in chapter 3, which suggests that the different diets have unique effects on oxylipin metabolism in females and males.

Males have higher oxylipin levels than females in most cases and almost all of the enzymes with a sex effect have higher product/substrate levels in males. Thus even though the liver AA level was higher in females, for example, almost all AA derived oxylipins were higher in males. In the case of DHA oxylipins in kidney, however, the higher DHA level was associated with higher DHA oxylipins, even though the product/substrate ratio was higher or no different in males, indicating that the fatty acid

substrate level also influences oxylipin levels. To conclude, both conversion rate and fatty acid precursor levels may contribute to the differences in oxylipin levels in males and females.

There is also a sex effect for n-3 fatty acid conversion of ALA to DHA. A number of studies have shown that female rats have greater DHA levels than males (80, 81). Another study of lipid composition in blood of 1246 males and 1547 females eating their standard diets found higher levels of DHA in serum PL and cholesterol ester in women compared to men (82). The increased DHA fatty acid level may be due to the increased estrogen levels that are correlated with greater DHA presence (83-85). In the current study, DHA fatty acid levels are higher in EPA, ALA and control group in kidney and control group in liver, which is consistent with the previous research that females have higher levels of DHA.

4.4.7 Fatty Acid Incorporation And Enzyme Selectivity

Selectivity of oxylipin levels occurs not only with sex, but also with different fatty acids (Table 4. 10. a-f). When the product/substrate ratios for enzymes in several oxylipin biosynthetic pathways were examined, these ratios indicated a higher selectivity for n-3 fatty acid over n-6 fatty acid conversion to their respective metabolites. Similar findings were shown in obese rats (43), and in studies of CYP selectivity of product formation from EPA and DHA compared to AA (49, 86, 87). These findings and the fact that a higher proportion of ALA is metabolized via β-oxidation (88), may partly explain why ALA levels are lower than LA in tissues. Additionally, these ratios show that these enzymes convert C18 fatty acids more readily than their longer chains fatty acid counterparts, consistent with chapter 3. Previous findings also show that enzymes such as elongase and LOX prefer to metabolize shorter chain fatty acid to longer chain (89, 90) and. However the results were not as consistent in the n-3 fatty acid groups. For example, in the ALA and DHA groups, kidney DHA oxylipin/ DHA was higher than EPA

oxylipin/EPA; and in the EPA group, serum DGLA oxylipin/DGLA was higher than LA oxylipin/LA. This suggests that dietary fatty acid levels also may influence the apparent rate of conversion of fatty acid to oxylipins. This remains to be elucidated.

To conclude, dietary ALA is sufficient to increase DHA oxylipins without altering DHA levels, but direct feeding of DHA is more effective in this regard. EPA and DHA have unique effects on the n-3 oxylipin profile. Each one most strongly modulates the levels of its own oxylipins, but alterations of other n-3 fatty acid derived oxylipins provide evidence for conversion and retro conversion of these fatty acids. ALA, EPA and DHA all can decrease n-6 oxylipins; DHA was more potent than EPA than ALA in this regard. Most oxylipins and product/substrate ratios were higher in males. In most cases, enzymes convert more C18 fatty acid than C20 than C22, and more n-3 fatty acid than n-6 fatty acid. These data provide novel perspectives on fatty acid metabolism that may have implications for intake of dietary n-3 fatty acids.

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Chapter 5 Thesis Discussion

5.1 Thesis Discussion

There are controversies of LA, ALA, EPA and DHA intake. Regarding these debates, the current study examined the effects of dietary LA, ALA, EPA and DHA on oxylipin production. It provided evidence that with a higher LA intake, LA, AA and many other n-6 derived oxylipins are increased and that n-3 derived oxylipins are decreased. High LA intake increased AA oxylipins, but AA level did not change. This is consistent with other research showing that the fatty acid level does not always reflect oxylipin levels (1, 2). The same discrepancy between fatty acid and oxylipin levels was also observed in the sex effect on fatty acids and oxylipins. This suggests that fatty acid level may not represent oxylipin levels.

For n-3 fatty acid groups, high ALA intake increased DHA oxylipins without increasing DHA levels, which is consistent with a diseased kidney model which showed that ALA feeding increased DHA oxylipins without a higher DHA level (3). This suggests that even if the conversion of ALA to DHA fatty acid is low, ALA can be converted to DHA oxylipins. With high EPA and high DHA feeding, n-3 derived oxylipins are significantly increased by ~210%-1900% with a decrease in n-6 oxylipins. This is consistent with previous research which showed that with n-3 fatty acid feeding, n-3 derived oxylipins are significantly elevated and n-6 oxylipins are decreased (4-6); however, there is no research has investigated the effect of EPA alone or DHA alone on oxylipin levels. When comparing the effects of EPA alone and DHA alone, which are documented to have different effects on vascular function and inflammation (7, 8), the current result showed that DHA decreased more mass and number of AA oxylipins. When comparing EPA and DHA with ALA, EPA and DHA groups resulted in lower AA oxylipins. DHA also could be a retroconverted to EPA oxylipins, which is consistent with (9) which showed an increased level of EPA oxylipins after DHA intake in piglet plasma.

The different oxylipin profile in the EPA and DHA groups may also help explain the different effects of EPA and DHA on disease status effects such as inflammation.

With respect to sex effects on oxylipins, most oxylipins are higher in males with few exceptions such as DHA derived oxylipins in kidney. The product/precursor ratio is also higher in males. Product/precursor ratios showed that DHA was converted to DHA oxylipins more in males, suggesting that the higher kidney DHA oxylipins in females was due to the higher DHA levels in female kidneys. Both substrate level and enzyme conversion rate can affect oxylipin production. In the current study, the formation of oxylipins from n-3 fatty acids were generally greater than from n-6 fatty acids, which is consistent with (10) which showed that CYP enzymes convert n-3 fatty acids to oxylipins more than n-6 fatty acids. The formation of oxylipins from C18 fatty acids was generally greater than C20 than C22 fatty acid as well; this is consistent with previous research (11) that showed the oxylipin production from C18 is greater than C20 and C22.

5.2 Conclusions

These results illustrate that although dietary LA may not alter the concentrations of LA and AA, it does increase the levels of bioactive lipids derived from both LA and AA in rat tissues. Similarly, sex effects on oxylipins are not reflected in fatty acid levels in these tissues. Adding ALA can mitigate some of the high LA effects.

This study also illustrates that although dietary ALA may not alter tissue DHA concentrations, it does increase the levels of many oxylipins derived from DHA in kidney and serum. DHA, EPA and ALA have unique effects on n-3 derived oxylipins. All these n-3 fatty acids can decrease n-6 oxylipins, with DHA having the greatest effect.

5.3 Strengths

1. The advanced methods of the current study allowed for analysis of oxylipin profile up to 164 oxylipins scanned and generally 80 oxylipins were detected in each tissue. Oxylipins are from various precursors including ADA, EDA, DGLA etc.
2. The varying levels of LA, ALA, EPA and DHA provided excellent comparisons to understand how dietary fatty acids can affect oxylipin profiles.
3. It is the first study to look at sex effects on the oxylipin profile in any species or tissues.
4. The diets allowed us to make comparisons of high LA alone and high LA+ALA, the latter one mimics normal diets, which is the AIN93 for laboratory rodents and indicated high LA+ALA will also increase AA oxylipins compared to an adequate LA diet.
5. This study used individual EPA and DHA rich fish oils, which allowed us to differentiate the individual EPA and DHA effects on oxylipin production for the first time.
6. This is also the first study to investigate the effect of high ALA intake on the oxylipin profile.

5.4 Limitations

1. With oil composition, the MUFA in LA+ALA group, high LA group, ALA group, EPA group and DHA group is not consistent, ranging from 0.92-1.95g/100g in the diets, which creates a variable for the study design. MUFA is not reported to produce oxylipins so far, but there is also no evidence that MUFA is not able to produce oxylipins. So it is desirable to keep MUFA consistent in each group.

2. Only free oxylipins are analyzed, so there is a lack of esterified oxylipin measurement. There is no information on how the changes in esterified oxylipins were altered by different fatty acid intake, and there is also no information on how the changes in esterified and free oxylipins are correlated.
3. The ratio of product/precursor was measured to estimate the rate of conversion of fatty acids to oxylipins; however the enzymes were not measured directly, so the enzyme amount, activity and selectivity were not determined.
4. This study did not examine any effects on physiological functions.
5. Only one level of high LA, ALA, EPA and DHA was used in this study so that we did not know the effect of the level of fatty acid on oxylipins. For example, whether higher than 20%E of fatty acid can produce even more oxylipins, or doses lower than 4%E of fatty acid can produce even less oxylipins.
6. The 5 week body weight and weight gain of rats in chapter 3 is different; the 3, 4, 6 week body weight and weight gain of rats in chapter 4 is different. The relationship between body weight and oxylipin production is unclear, so that the different body weight is a limitation.

5.5 Implications

Dietary advice to increase LA intake may need to be re-considered in light of its effect on the production of some pro-inflammatory bioactive lipids from both LA and AA. The tripling of U.S. per capita average dietary LA from about 2%E to 7%E during the 20th century (12) may contribute to an increase in AA oxylipins. So the effect of a high intake of LA on increased AA derived oxylipins which are most pro inflammatory should be considered.

Regarding ALA, EPA and DHA intake, our current study indicated that higher ALA

intake increases DHA oxylipins, and that EPA alters oxylipin profile differently than DHA does. DHA has stronger effects on decreasing n-6 oxylipins. These findings may be relevant to n-3 fatty acid intake recommendations to differentiate EPA and DHA, at least to make people to consider them separately. ALA can be converted to DHA oxylipins, so organizations may consider whether DHA can be replaced with (some) ALA.

The tissue oxylipin profile is different than the serum oxylipin profile, which indicates that the information from human clinical trials that use serum analysis may not accurately represent other tissues.

The identification of the oxylipin profile and the influence of dietary LA, ALA, EPA and DHA improved the knowledge on lipid biochemistry and potential dietary fatty acid recommendations. This also helped to understand the fatty acid effect through alteration of oxylipins.

5.6 Future Directions

1. Clinical trials can be done to investigate the effect of dietary fatty acid in humans to determine whether the effect on oxylipins production is similar to rodents.
2. The fatty acid level can be reduced to lower than 5% and increased up to 20% to see the effect of different levels of fat on oxylipin production.
3. The effect of n-3 fatty acids on oxylipin profiles in disease models can be analyzed to investigate the oxylipin effect on inflammation, heart disease, mental health, immune disorders and so forth.
4. LA oxylipins and its physiological roles need to by fully investigated, for example LA oxylipins and cardiovascular disease, the relationship between LA oxylipins and inflammation as a mechanism of disease pathogenesis.
5. Diets low in LA should be analyzed to confirm whether reducing AA derived

oxylipins results in a reduction in inflammation. The effect of diet low in LA can be tested on some diseased models such as heart disease patients. Vice versa, the potential effect of high LA and low ALA should be confirmed in human clinical trials. The current study showed that high LA with adequate ALA increased many AA oxylipins. Most AA oxylipins have proinflammatory and vasoconstrictory effects, so it needs to be confirmed whether high LA with adequate ALA diet have negative effects on health.

6. Different combinations of LA and ALA in the diet can be analyzed in disease states. For example, the effect of low LA and high ALA, high LA and high ALA, low LA and low ALA on oxylipins in diseases such as chronic heart disease.
7. Clinical trials which only contains LA as the only variable that change, for example, the relationship between different LA levels and its pro inflammatory effects.
8. Comparison of the effect of pure EPA and pure DHA to a mixture of EPA and DHA on some disease status and oxylipin profiles to differentiate their effects.
9. There is a lack of understanding ALA and its oxylipin functions, ALA oxylipins can be investigated in cells to confirm their physiological and pathological effects and human clinical trials can be done to confirm ALA oxylipin effects and their relationship to disease status.

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Appendices

Appendix 1

Appendix 1.1. Fatty Acid Composition In Oils

	Soy	Safflower	Coconut	Flax	Olive	EPA	DHA
				g fatty acid/100g oil			
C40	0.357	0.392	0.341	0	0	0	0.356
C60	0	0	0.39	0	0	0	0
C80	0	0	5.26	0	0	0	0
C100	0	0	4.38	0	0	0	0
C120	0.00768	0	35.5	0.00846	0	0	0
C140	0.0687	0.101	14.1	0.0453	0	0	0
C150	0.0411	0.0269	0	0.0307	0	0	0.0129
C160	10.5	6.36	8.23	5.12	13.2	0.0434	0.024
C161	0.105	0.109	0	0.0881	1.67	0	0
C180	3.73	2.67	2.36	3.34	2.14	0.0333	0.0163
C181	20.4	17.06	6.43	17	50.9	0.148	0.0479
C182n6	53.5	73	1.67	16.2	13.1	0.252	0.0658
C183n6	0.00875	0	0	0.05	0	0.0717	0
C183n3	7.14	0.301	0	52.1	0.552	0.079	0.0284
C200	0.364	0.356	0	0.142	0.373	0	0
C201	0.194	0.203	0	0.217	0.212	0.166	0
C202n6	0.0461	0.0283	0	0.0379	0	0.0486	0
C203n6	0	0	0	0.0185	0	0.295	0
C204n6	0.0257	0.0145	0	0.0386	0.0207	2.48	0.00738
C203n3	0.0373	0.0163	0	0.0813	0	0.0571	0
C205n3	0	0.0144	0	0	0	95.3	0.341
C220	0.449	0.247	0	0.128	0.115	0	0
C221	0	0.0223	0	0.0668	0	0.0483	0.0514
C222n6	0	0	0	0	0	0	0.042
C224n6	0.0264	0	0	0	0	0	1.85
C225n3	0.0204	0	0	0.0273	0	0.0736	0
C226n3	0	0	0	0	0	0.432	95.1
C240	0.131	0.119	0	0.0788	0.0585	0	0
C241	0	0.147	0	0	0	0	0

Values are mean of duplicates

Appendix 1.2.A Oxylipins Scanned But Not Detected In Chapter 1

In kidney:

15deoxy-PGA_2; 15k-PGD_2; dhk-PGD_2; dihomo 15deoxy-PGD_2; 6k-PGE_1; 19 OH PGE_2; 20oh PGE_2; dhk-PGE_2; bicycle PGE_2; 11β-PGE2; 15k-$PGF_{1\alpha}$; dh-$PGF_{2\alpha}$; 19oh $PGF_{2\alpha}$; 20oh $PGF_{2\alpha}$; 11β-$PGF_{2\alpha}$; 2,3-dinor-8-iso-$PGF_{2\alpha}$; 15K$PGF_{2\alpha}$; dhk-$PGF_{2\alpha}$; 11β-$PGF_{2\alpha}$; 2,3dinor-6k-$PGF_{1\alpha}$; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; PGJ_2; dihomo PGJ_2; 2,3-dinor; PGK_2; PGK_1; 2,3-dinor-TXB_2; 11d-TXB_2; HXA_3; 20oh LTB_4; 20 COOH LTB_4;12epi-LTB_4; 6t,-12epi-LTB_4; 12oxo-LTB_4; LTC_4; LTD_4; LTE_4; 6R-LXA_4; 6S-LXA_4; 15R-LXA_4; 14,15-LTC_4 (EXC_4); 14,15-LTD_4 (EXD_4); 14,15-LTE_4 (EXE_4); 6R-LXA_4; LXB_4; 20 COOH AA; 5,6-diHETE; 5,6-EpETrE; 8,9-EpETrE; 11,12-EpETrE; 14,15-EpETrE; 17-HETE; 19-HETE; 17k-DPA; 9,10,13-triHOME; TXB_1; PGD_1; 9,10-EpOME; 12,13-EpOME; 12,13-EpODE; PGD_3; 11-HEPE; RvE_1; 14,15 EpETE; 17,18-EpETE; 16,17-EpDPE; 19,20-EpDPE; 7R Maresin-1; RvD_1; RvD_2; 17k-DHA; 9-Nitrooleate; 10-Nitrooleate;

In liver:

15deoxy-PGA_2; PGB_2; 12-HHTrE; HXA_3; HXB_3; 15k-PGD_2; dhk-PGD_2; 19oh PGE_2; 20oh PGE_2; 15k-PGE_2; 11β-PGE_2; dh-PGE_2; dh-$PGF_{2\alpha}$; 19oh $PGF_{2\alpha}$; 20oh $PGF_{2\alpha}$; 2,3-dinor 11β-$PGF_{2\alpha}$; 11β-$PGF_{2\alpha}$; 15k $PGF_{2\alpha}$; 11β-dhk-$PGF_{2\alpha}$; PGJ_2; PGK_2; 2,3-dinor TXB_2; 11d-TXB_2; 20 COOH AA; 20oh LTB4; 20 COOH LTB_4; 6t-LTB_4; 12epi-LTB_4; 6t, 12epi-LTB_4; 12oxo-LTB_4; LTC_4; LTD_4; LTE_4; 14,15-LTC_4 (EXC_4); 14,15-LTD_4 (EXD_4); 14,15-LTE_4 (EXE_4); 6R-LXA_4; 15R-LXA_4; LXA_5; 6S-LXA_4; LXB_4; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; 5,15-DiHETE; 5,6 diHETE; 5,6-EpETrE; 11,12-EpETrE; 14,15-EpETrE; 14,15-EpETE; 17,18-EpETE; 8-iso 15k $PGF_{2\beta}$; 2,3-dinor 8-iso-$PGF_{2\alpha}$; dihomo 15deoxy-PGD_2; dihomo PGE_2; dihomo PGJ_2; 9,10,13-triHOME; PGD_1; PGE_1; 6k-PGE_1; 6,15-dk-,dh-$PGF_{1\alpha}$; 15k-$PGF_{1\alpha}$; Δ^{17}-6k-PGF1α; 2,3-dinor-6k-$PGF_{1\alpha}$; PGK_1; TXB_1; 9,10-EpODE; 15,16-diHODE; 9,10-diHODE; 12,13-diHODE ; 12,13-EpODE; 15,16-EpODE; 13-oxoOTrE; $PGF_{3\alpha}$; PGD_3;11-HEPE; 8-iso $PGF_{3\alpha}$; R Maresin-1; 16,17-EpDPE; RvD_2; RvD_1; PD_1; 15t-PD_1; 17k-DHA;17k-DPA; 9-Nitrooleate; 10-Nitrooleate;

In Serum:

PGA_2; 15deoxy-PGA_2; PGB_2; 15k-PGD_2; dhk-PGD_2; 15deoxy-PGD_2; 20oh PGE_2; dhk-PGE_2; 11β-PGE_2; PGK_2; bicyclo PGE_2; 19oh PGE_2; 15k-$PGF_{2\alpha}$; dh-$PGF_{2\alpha}$; 11β-dhk-$PGF_{2\alpha}$; 19oh $PGF_{2\alpha}$; 20oh $PGF_{2\alpha}$; 2,3-dinor, 11β-$PGF_{2\alpha}$; 15k-$PGF_{2\beta}$; dhk-PGF_{2a}; 20oh LTB_4; 20 COOH LTB_4;

12epi-LTB_4; LXB_4; 6t-LTB_4; 12epi-LTB_4; 12oxo-LTB_4; LTC_4; LTD_4; LTE_4; 14,15-LTC_4(EXC_4); 14,15-LTD_4(EXD_4); 14,15-LTE_4(EXE_4); 6R-LXA_4; 6S-LXA_4; 15R-LXA_4; LXA_5; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; 2,3-dinor TXB_2; 11d-TXB_2;5,6 diHETE; 5,6 EpETrE; 14,15 EpETE; 12-HHTrE; 16-HETE; 19-HETE; HXA_3; 20 COOH AA; 8-iso $PGF_{2\alpha}$III; 2,3-dinor 8-iso $PGF_{2\alpha}$; 5-HETrE; dihomo PGD_2; dihomo 15deoxy PGD_2; dihomo PGE_2; dihomo $PGF_{2\alpha}$; 2,3-dinor-6k $PGF_{1\alpha}$; TXB_1; PGE_1; 6,15-dk-,dh-$PGF_{1\alpha}$; 15k-$PGF_{1\alpha}$; PGK_1; 8-iso $PGF_{3\alpha}$; 15-oxoEDE; 9,10,13-triHOME; 9,10-diHODE; 12,13-diHODE; 15,16-EpODE; 13-oxoOTrE 9,10-EpODE; 15,16-diHODE; PGD_3; 11-HEPE; 9-HEPE; RvE_1; RvD_1; 15t-PD_1; RvD_2; 7R Maresin-1; PD_1; PDX; 17k-DHA; 17k-DPA; 9-Nitrooleate; 10-Nitrooleate;

Appendix 1.2.B: Oxylipins Scanned But Not Detected In Tissues In Chapter 2

In kidney:

11β-$PGF_{2\alpha}$; 11β-PGE_2; 15deoxy-PGA_2; 15k-PGD_2; 15k-$PGF_{2\alpha}$; 19oh PGE_2; 19oh $PGF_{2\alpha}$; 2,3-dinor 11β-$PGF_{2\alpha}$; 2,3-dinor TXB_2; 2,3-dinor-6k $PGF_{1\alpha}$; 20oh PGE_2; 20oh $PGF_{2\alpha}$; bicyclo PGE_2; dh-$PGF_{2\alpha}$; dhk-PGD_2; dhk-PGE_2; dhk-$PGF_{2\alpha}$; PGB_2; PGJ_2; PGK_2; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; 12epi-LTB_4; 12oxo-LTB_4; 14,15-LTC_4 (EXC_4); 14,15-LTD_4 (EXD_4); 14,15-LTE_4 (EXE_4); 15R-LXA_4; 20 COOH- LTB_4; 20oh LTB_4; 5,6-DiHETE; 6R-LXA_4; 6S-LXA_4; 6t, 12epi-LTB_4; HXA_3; LTC_4; LTD_4; LTE_4; LXB_4; 11,12-EpETrE; 14,15-EpETrE; 17-HETE; 19-HETE; 20 COOH AA; 5,6-EpETrE; 8,9-EpETrE; 2,3-dinor 8-iso $PGF_{2\alpha}$; dihomo 15deoxy PGD_2; dihomo PGJ_2; 19,20-EpDPE; 10S,17S-DiHDoHE (PDX); 15t-PD_1; 7R Maresin-1; PD_1; RvD_2; RvD5; 16,17-EpDPE; 17k DPA; 15k-$PGF_{1\alpha}$; 6k-PGE_1; PGD_1; PGK_1; TXB_1; PGD_3; 14,15-EpETE; 17,18-EpETE; RvD_1; RvE_1; 11-HEPE; 12,13-EpODE; 12,13-EpOME; 15,16-DiHODE; 15,16-EpODE; 9,10-DiHODE; 9,10-EpODE; 9,10-EpOME; 9,10,13-TriHOME; 5-HETrE; 10-Nitrooleate; 9-Nitrooleate.

In liver:

11β-dhk $PGF_{2\alpha}$; 11d-TXB_2; 11β-$PGF_{2\alpha}$; 11β-PGE_2; 12-HHTrE; 15deoxy-PGA_2; 15k-PGD_2; 15k-PGE_2; 15k-$PGF_{2\alpha}$; 19oh PGE_2; 19oh $PGF_{2\alpha}$; 2,3-dinor 11β-$PGF_{2\alpha}$; 2,3-dinor TXB_2; 2,3-dinor-6k $PGF_{1\alpha}$; 20oh PGE_2; 20oh $PGF_{2\alpha}$; dh-$PGF_{2\alpha}$; dhk-PGD_2; dhk-PGE_2; dhk-$PGF_{2\alpha}$; PGB_2; PGJ_2; PGK_2; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; 12epi LTB_4; 12oxo LTB_4; 14,15-LTC_4 (EXC_4); 14,15-LTD_4 (EXD_4); 14,15-LTE_4 (EXE_4); 15R-LXA_4; 20 COOH LTB_4; 20oh LTB_4; 5,15-DiHETE; 5,6-DiHETE; 6R-LXA_4; 6S-LXA_4; 6t LTB_4; 6t, 12epi LTB_4; HXA_3; HXB_3; LTC_4; LTD_4; LTE_4; LXB_4; 2,3-dinor 8-iso $PGF_{2\alpha}$; 8-iso 15k $PGF_{2\beta}$; 11,12-EpETrE; 14,15-EpETrE; 20 COOH AA; 5,6-EpETrE; dihomo 15deoxy PGD_2; dihomo PGE_2; dihomo PGJ_2; 13-oxoOTrE; 9-HOTrE; 16,17-EpDPE; 10S,17S-DiHDoHE (PDX); 15t-PD_1; 17k DHA; 7R Maresin-1; PD_1; RvD_2; RvD_5; 17k-DPA; 15k-$PGF_{1\alpha}$; 6,15-dk-dh-$PGF_{1\alpha}$; 6k-PGE_1; PGD_1; PGE_1; PGK_1; TXB_1; Δ^{17}-6k-PGF1α; PGD_3; $PGF_{3\alpha}$; LXA_5; RvD_1; 14,15-EpETE; 17,18-EpETE; 8-iso $PGF_{3\alpha}$; 11-HEPE; 12,13-DiHODE; 12,13-EpODE; 15,16-DiHODE; 15,16-EpODE; 9,10-DiHODE; 9,10-EpODE; 9,10,13-TriHOME; 10-Nitrooleate; 9-Nitrooleate.

In Serum:

11β-dhk-$PGF_{2\alpha}$; 11d-TXB_2; 11β-$PGF_{2\alpha}$; 11β-PGE_2; 12-HHTrE; 15deoxy-PGA_2; 15deoxy-PGD_2; 15k-PGD_2; 15k-$PGF_{2\alpha}$; 19oh PGE_2; 19oh $PGF_{2\alpha}$; 2,3-dinor 11β $PGF_{2\alpha}$; 2,3-dinor TXB_2; 2,3-dinor-6k $PGF_{1\alpha}$; 20oh PGE_2; 20oh $PGF_{2\alpha}$; bicyclo PGE_2; dh-$PGF_{2\alpha}$; dhk-PGD_2; dhk-PGE_2; dhk-$PGF_{2\alpha}$; PGA_2; PGB_2; PGK_2; tetranor-PGDM; tetranor-PGEM; tetranor-PGFM; 12epi-LTB_4; 12oxo-LTB_4; 14,15-LTC_4 (EXC_4); 14,15-LTD_4 (EXD_4); 14,15-LTE_4 (EXE_4); 15R-LXA_4; 20 COOH LTB_4; 20oh LTB_4; 5,6-DiHETE; 6R-LXA_4; 6S-LXA_4; 6t-LTB_4; 6t, 12epi-LTB_4; HXA_3; LTC_4; LTD_4; LTE_4; LXB_4; 16-HETE; 19-HETE; 20 COOH AA; 5,6-EpETrE; 2,3-dinor 8-iso $PGF_{2\alpha}$; 5-iso $PGF_{2\alpha}$VI; 8-iso 15k $PGF_{2\beta}$; 8-iso $PGF_{2\alpha}$III; dihomo 15deoxy PGD_2; dihomo PGD_2; dihomo PGE_2; dihomo $PGF_{2\alpha}$; dihomo PGJ_2; 13-oxoOTrE; 9-HOTrE; 10S,17S-DiHDoHE (PDX); 15t PD_1; 17k DHA; 7R Maresin-1; PD_1; RvD_2; RvD_5; 17k-DPA; 15k-$PGF_{1\alpha}$; 6,15-dk-dh-$PGF_{1\alpha}$; 6k-PGE_1; PGE_1; PGK_1; TXB_1; Δ^{17}-6k-PGF1α; PGD_3; 14,15-EpETE; 15-oxoEDE; LXA_5; RvD_1; RvE_1; 8-iso $PGF_{3\alpha}$; 11-HEPE; 9-HEPE; 12,13-EpODE; 15,16-DiHODE; 15,16-EpODE; 9,10-DiHODE; 9,10-EpODE; 9,10,13-TriHOME; 5-HETrE; 10-Nitrooleate; 9-Nitrooleate.

Appendix 1.3: Oxylipins Scanned For That Did Not Have A Primary Standard

Component Name	Dumlao RT[#]	Estimated Primary RT*
HXA_3	14.3	**14.6**
HXB_3	14.4	**14.8**
LTD_4	10.6	**9.19**
LTE_4	10.2	**10.57**
14,15-LTC4 (EXC_4)	7.2	**7.5**
14,15-LTD4 (EXD_4)	10.7	**10.98**
14,15-LTE4 (EXE_4)	8.7	**9**
15R-LXA_4	8.1 (W) ❖	**8.5**
15k-PGD_2	7.7 (W)	**8.64**
20oh PGE_2	3.5	**3.15**
dh-$PGF_{2\alpha}$	7.7	**7.97**
19oh $PGF_{2\alpha}$	3.3	**2.95**
20oh $PGF_{2\alpha}$	3.2	**2.85**
tetranor-PGDM	4.5 (W)	**2.39**
tetranor-PGFM	2.5	**2.3**
9,10-EpODE		**16.39**
9,10-DiHODE		**11.62**
15,16-EpODE		**15.49**
15,16-DiHODE		**10.62**
15k-$PGF_{1\alpha}$	7.4	**7.68**
dihomo PGE_2	9.1	**9.4**
dihomo PGD_2	9.4	**9.7**
dihomo PGJ_2	12.3	**12.52**
dihomo 15deoxy PGD_2	13.9	**14.08**
8-iso $PGF_{3\alpha}$	5.3 (W)	**5.5**
PD_1	10.5	**10.8**
15t-PD_1	10.8	**11**
9-Nitrooleate	18.1 (W)	**19.65**
10-Nitrooleate	18.1 (W)	**19.65**

Dumlao RT refers to the retention time in the following published paper:
Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA. High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acylethanolamines. BBA-Mol Cell Biol L 2011;1811(11):724-36. doi: 10.1016/j.bbalip.2011.06.005.

* The Estimated primary RT is the estimated retention time of the oxylipin, based on known and unknown retention time of oxylipins in our samples and the retention times of oxylipins reported by Dumlao et al, 2011 (above footnote).

❖ Indicates that there is no retention time in published paper by Dumlao et al., so that this number is estimated from Wang Y, Armando AM, Quehenberger O, Yan C, Dennis EA. Comprehensive ultra-performance liquid chromatographic separation and mass spectrometric analysis of eicosanoid metabolites in human samples. Journal of Chromatography A 2014;1359:60-9. doi: 10.1016/j.chroma.2014.07.006.

Appendix 1.4: List Of Mass Transitions, Retention Times, Deuterated Internal Standards And Dose Response Factors For All The Oxylipins

Component Name	Mass Transition	Retention Time	Deuterated Internal Standard	Dose Response Factor
12-HHTrE	279.0 / 217.0	13.53	(d8) 5-HETE.IS	0.3646
PGA_2	333.0 / 271.0	9.68	(d4) 15deoxy PGJ_2.IS	11.454
15deoxy-PGA_2	315.0 / 271.0	15.19	(d4) 15deoxy PGJ_2.IS	2.6609
PGB_2	333.0 / 271.0	12.29	(d4) 15deoxy PGJ_2.IS	1.0891
PGD_2	351.0 / 271.0	7.78	(d4) PGD_2.IS	1.6144
15deoxy -PGD_2	333.0 / 271.0	11.81	(d4) 15deoxy PGJ_2.IS	29.722
dhk-PGD_2	351.0 / 207.0	9.15	(d4) PGD_2.IS	2.34
15k-PGD_2	349.0 / 235.0	*	(d4) PGD_2.IS	No Primary
tetranor-PGDM	327.0 / 247.0	2.39	(d4) PGD_2.IS	0.0984
PGE_2	351.0 / 271.0	7.43	(d4) PGE_2.IS	7.8596
11β-PGE_2	351.0 / 271.0	7.58	(d4) PGE_2.IS	4.9206
19oh-PGE_2	367.0 / 243.0	3.25	(d4) PGE_2.IS	0.3835
bicyclo-PGE_2	333.0 / 175.0	11.00 10.17	(d4) PGE_2.IS	0.7943
15k-PGE_2	349.0 / 235.0	7.78	(d4) PGE_2.IS	1.3377
20oh-PGE_2	367.0 / 175.0	*	(d4) PGE_2.IS	No Primary
dhk-PGE_2	351.0 / 207.0	8.44	(d4) PGE_2.IS	0.529
tetranor-PGEM	327.0 / 291.0	2.3	(d4) PGE_2.IS	0.4032
$PGF_{2\alpha}$	353.0 / 193.0	7.25	(d4) $PGF_{2\alpha}$.IS	3.4181
dhk-$PGF_{2\alpha}$	353.0 / 291.0	8.64	(d4) dhk $PGF_{2\alpha}$.IS	4.9299
dh-$PGF_{2\alpha}$	355.0 / 283.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
19oh-$PGF_{2\alpha}$	369.0 / 192.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
20oh-$PGF_{2\alpha}$	369.0 / 165.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
11β dhk-$PGF_{2\alpha}$	353.0 / 113.0	8.2	(d4) $PGF_{2\alpha}$.IS	0.8673
11β-$PGF_{2\alpha}$	353.0 / 335.0	6.74	(d4) $PGF_{2\alpha}$.IS	0.1703
15k-$PGF_{2\alpha}$	351.0 / 219.0	7.68	(d4) $PGF_{2\alpha}$.IS	0.6609
6k-$PGF_{1\alpha}$	369.0 / 245.0	5.34	(d4) 6k $PGF_{1\alpha}$.IS	3.6495
2,3-dinor-6k-$PGF_{1\alpha}$	363.0 / 281.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
2,3-dinor-11β-$PGF_{2\alpha}$	325.0 / 227.0	5.4	(d4) $PGF_{2\alpha}$.IS	1.2716
tetranor-PGFM	329.0 / 247.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
PGJ_2	333.0 / 189.0	9.87	(d4) 15deoxy PGJ_2.IS	3.6267
15deoxy-PGJ_2	315.0 / 203.0	14.57	(d4) 15deoxy PGJ_2.IS	1.4436
PGK_2	349.0 / 249.0	7.71	(d4) PGE_2.IS	1.8211
TXB_2	369.0 / 169.0	6.61	(d4) TXB_2.IS	6.3658
11d-TXB_2	367.0 / 305.0	7.39	(d4) TXB_2.IS	2.375
2,3-dinor-TXB_2	341.0 / 137.0	5.22	(d4) TXB_2.IS	0.3628
5-HETE	319.0 / 115.0	17.19	(d8) 5-HETE.IS	1.169
5-oxoETE	317.0 / 203.0	17.56	(d7) 5-oxoETE.IS	0.8498
5,15-DiHETE	335.0 / 201.0	11.46	(d4) LTB_4.IS	0.5267
5,6-DiHETE	335.0 / 115.0	15.29	(d4) LTB_4.IS	0.4905
8-HETE	319.0 / 155.0	16.71	(d8) 5-HETE.IS	2.311
8,15-DiHETE	335.0 / 235.0	10.98	(d4) LTB_4.IS	0.1268
9-HETE	319.0 / 123.0	16.84	(d8) 5-HETE.IS	0.2492
11-HETE	319.0 / 167.0	16.41	(d8) 5-HETE.IS	5.3158
12-HETE	319.0 / 135.0	16.6	(d8) 15-HETE.IS	0.189
tetranor 12-HETE	265.0 / 109.0	13.28	(d8) 15-HETE.IS	2.0436
12-oxoETE	317.0 / 153.0	16.6	(d7) 5-oxoETE.IS	1.5173
15-HETE	319.0 / 175.0	16.07	(d8) 15-HETE.IS	0.9018
15-oxoETE	317.0 / 113.0	16.14	(d7) 5-oxoETE.IS	2.2341
HXA_3	335.0 / 195.0	*	(d4) LTB_4.IS	No Primary
HXB_3	335.0 / 183.0	*	(d4) LTB_4.IS	No Primary
LTB_4	335.0 / 195.0	11.93	(d4) LTB_4.IS	1.2507
6t-LTB_4	335.0 / 195.0	11.44	(d4) LTB_4.IS	0.9015
6t, 12epi-LTB_4	335.0 / 195.0	11.58	(d4) LTB_4.IS	0.6674
12epi-LTB_4	335.0 / 195.0	11.89	(d4) LTB_4.IS	1.4375
12oxo-LTB_4	333.0 / 179.0	12.6	(d4) LTB_4.IS	1.4903
20 COOH LTB_4	365.0 / 195.0	5.35	(d4) LTB_4.IS	0.1691

20oh LTB_4	351.0 / 195.0	5.6	(d4) LTB_4.IS	0.6127
LTC_4	624.0 / 272.0	10.75	(d4) LTB_4.IS	0.3364
14,15-LTC_4 (EXC_4)	624.0 / 272.0	*	(d4) LTB_4.IS	No Primary
LTD_4	495.0 / 177.0	9.19	(d4) LTB_4.IS	1.0476
14,15-LTD_4 (EXD_4)	495.0 / 177.0	*	(d4) LTB_4.IS	No Primary
LTE_4	438.0 / 333.0	10.57	(d4) LTB_4.IS	0.9368
14,15-LTE_4 (EXE_4)	438.0 / 333.0	*	(d4) LTB_4.IS	No Primary
15R-LXA_4	351.0 / 165.0	*	(d4) LTB_4.IS	No Primary
6R-LXA_4	351.0 / 217.0	8.5	(d4) LTB_4.IS	0.1535
6S-LXA_4	351.0 / 115.0	8.78	(d4) LTB_4.IS	0.292
LXB_4	351.0 / 221.0	7.58	(d4) LTB_4.IS	0.3733
20 COOH-AA	333.0 / 271.0	14.38	(d5) EPA.IS	1.1049
5,6-EpETrE	319.0 / 191.0	18.13	(d11) 11,12 DiHETrE.IS	3.7275
5,6-DiHETrE	337.0 / 145.0	15.56	(d11) 11,12 DiHETrE.IS	3.6065
8,9-EpETrE	319.0 / 155.0	17.9	(d11) 8,9 DiHETrE.IS	1.2994
8,9-DiHETrE	337.0 / 127.0	14.85	(d11) 8,9 DiHETrE.IS	5.5366
11,12-EpETrE	319.0 / 167.0	17.7	(d11) 11,12 DiHETrE.IS	9.1336
11,12-DiHETrE	337.0 / 167.0	14.27	(d11) 11,12 DiHETrE.IS	16.4237
14,15-EpETrE	319.0 / 175.0	17.24	(d11) 14,15 DiHETrE.IS	1.0437
14,15-DiHETrE	337.0 / 207.0	13.54	(d11) 14,15 DiHETrE.IS	10.939
16-HETE	319.0 / 189.0	15.51	(d8) 15-HETE.IS	0.5383
17-HETE	319.0 / 247.0	15.4	(d8) 15-HETE.IS	3.0768
18-HETE	319.0 / 261.0	15.28	(d6) 20-HETE.IS	3.2074
19-HETE	319.0 / 231.0	14.89	(d6) 20-HETE.IS	0.2715
20-HETE	319.0 / 245.0	15.02	(d6) 20-HETE.IS	0.3998
2,3-dinor-8-iso-$PGF_{2\alpha}$	325.0 / 237.0	5.09	(d4) $PGF_{2\alpha}$.IS	5.3857
5-iso $PGF_{2\alpha}$VI	353.0 / 115.0	7.02	(d4) $PGF_{2\alpha}$.IS	1.5803
8-iso $PGF_{2\alpha}$III	353.0 / 193.0	6.5	(d4) $PGF_{2\alpha}$.IS	1.9211
8-iso-15k-$PGF_{2\beta}$	351.0 / 219.0	6.96	(d4) $PGF_{2\alpha}$.IS	3.6218
9-HODE	295.0 / 171.0	15.95	(d4) 9-HODE.IS	1.4704
9-oxoODE	293.0 / 185.0	16.28	(d7) 5-oxoETE.IS	1.4353
13-HODE	295.0 / 195.0	15.8	(d4) 13-HODE.IS	2.9546
13-oxoODE	293.0 / 167.0	15.96	(d7) 5-oxoETE.IS	0.2717
9,10,13-TriHOME	329.0 / 171.0	7.32	(d4) 9-HODE.IS	3.1287
9,12,13-TriHOME	329.0 / 211.0	7.19	(d4) 9-HODE.IS	6.1871
9,10-EpOME	295.0 / 171.0	17.41	(d4) 9,10 DiHOME.IS	3.7629
9,10-DiHOME	313.0 / 201.0	13.22	(d4) 9,10 DiHOME.IS	16.426
12,13-EpOME	295.0 / 195.0	17.21	(d4) 12,13 DiHOME.IS	10.516
12,13-DiHOME	313.0 / 183.0	12.66	(d4) 12,13 DiHOME.IS	11.798
15-oxoEDE	321.0 / 223.0	17.92	(d7) 5-oxoETE.IS	1.1612
13-HOTrE-γ	293.0 / 193.0	14.89	(d4) 13-HODE.IS	1.6998
PGD_1	353.0 / 235.0	7.74	(d4) PGD_2.IS	1.0006
PGE_1	353.0 / 235.0	7.67	(d4) PGE_2.IS	1.4269
6k-PGE_1	367.0 / 331.0	5.65	(d4) PGE_2.IS	0.9207
$PGF_{1\alpha}$	355.0 / 293.0	7.24	(d4) $PGF_{2\alpha}$.IS	3.0675
15k-$PGF_{1\alpha}$	353.0 / 221.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
6,15-dk-dh-$PGF_{1\alpha}$	369.0 / 267.0	6.52	(d4) $PGF_{2\alpha}$.IS	0.4899
PGK_1	351.0 / 251.0	7.76	(d4) PGD_2.IS	4.2079
TXB_1	371.0 / 171.0	6.33	(d4) TXB_2.IS	3.6782
5-HETrE	321.0 / 205.0	18.65	(d8) 5-HETE.IS	0.2746
8-HETrE	321.0 / 157.0	17.12	(d8) 5-HETE.IS	1.8141
15-HETrE	321.0 / 221.0	16.74	(d8) 15-HETE.IS	2.9424
dihomo PGD_2	379.0 / 299.0	*	(d4) PGD_2.IS	No Primary
dihomo 15deoxy PGD_2	361.0 / 299.0	*	(d4) 15deoxy PGJ_2.IS	No Primary
dihomo PGE_2	379.0 / 299.0	*	(d4) PGE_2.IS	No Primary
dihomo $PGF_{2\alpha}$	381.0 / 337.0	9.2	(d4) $PGF_{2\alpha}$.IS	2.812
dihomo PGJ_2	361.0 / 299.0	*	(d4) 15deoxy PGJ_2.IS	No Primary
9-HOTrE	293.0 / 171.0	14.46	(d4) 9-HODE.IS	2.006
9-oxoOTrE	291.0 / 185.0	14.98	(d7) 5-oxoETE.IS	2.2547
13-HOTrE	293.4 / 195.0	14.59	(d4) 13-HODE.IS	0.6216
13-oxoOTrE	291.0 / 247.0	14.8	(d7) 5-oxoETE.IS	0.0856
12,13-EpODE	293.0 / 183.0	16.19	(d4) 13-HODE.IS	2.4048
12,13-DiHODE	311.0 / 183.0	11.12	(d4) 12,13 DiHOME.IS	4.4379

15,16-EpODE	293.0 / 235.0	*	(d4) 12,13 DiHOME.IS	No Primary
15,16-DiHODE	311.0 / 223.0	*	(d4) 12,13 DiHOME.IS	No Primary
9,10-EpODE	293.0 / 171.0	*	(d4) 9,10 DiHOME.IS	No Primary
9,10-DiHODE	311.0 / 201.0	*	(d4) 9,10 DiHOME.IS	No Primary
PGD_3	349.0 / 269.0	6.79	(d4) PGD_2.IS	0.5356
PGE_3	349.0 / 269.0	6.52	(d4) PGE_2.IS	1.3408
$PGF_{3\alpha}$	351.0 / 193.0	6.27	(d4) $PGF_{2\alpha}$.IS	1.2284
TXB_3	367.0 / 169.0	5.73	(d4) TXB_2.IS	5.4993
Δ^{17}-6k-$PGF_{1\alpha}$	367.0 / 163.0	4.68	(d4) $PGF_{2\alpha}$.IS	2.0835
5-HEPE	317.0 / 115.0	15.81	(d8) 5-HETE.IS	1.1101
8-HEPE	317.0 / 155.0	15.34	(d8) 5-HETE.IS	1.1697
9-HEPE	317.0 / 149.0	15.48	(d8) 5-HETE.IS	0.6765
11-HEPE	317.0 / 215.0	*	(d8) 5-HETE.IS	No Primary
12-HEPE	317.0 / 179.0	15.33	(d8) 15-HETE.IS	1.7239
15-HEPE	317.0 / 219.0	15	(d8) 15-HETE.IS	1.8731
LXA_5	349.0 / 215.0	7.32	(d4) LTB_4.IS	0.3762
RvD_1	375.0 / 141.0	8.36	(d4) LTB_4.IS	0.5531
14,15-EpETE	317.0 / 207.0	16.27	(d11) 14,15 DiHETrE.IS	2.6835
17,18-EpETE	317.0 / 259.0	15.88	(d11) 14,15 DiHETrE.IS	1.8647
18-HEPE	317.0 / 215.0	14.5	(d6) 20-HETE.IS	1.3858
RvE_1	349.0 / 195.0	5.51	(d4) LTB_4.IS	0.2271
8-iso-$PGF_{3\alpha}$	351.0 / 307.0	*	(d4) $PGF_{2\alpha}$.IS	No Primary
17k-DHA	341.0 / 297.0	16.31	(d4) LTB_4.IS	1.1412
4-HDoHE	343.0 / 101.0	17.37	(d8) 5-HETE.IS	0.4409
7-HDoHE	343.0 / 141.0	16.7	(d8) 5-HETE.IS	0.5523
8-HDoHE	343.0 / 109.0	16.77	(d8) 5-HETE.IS	0.4476
10-HDoHE	343.0 / 153.0	16.36	(d8) 5-HETE.IS	2.1831
11-HDoHE	343.0 / 149.0	16.5	(d8) 5-HETE.IS	0.8501
13-HDoHE	343.0 / 221.0	16.18	(d8) 15-HETE.IS	0.603
14-HDoHE	343.0 / 205.0	16.3	(d8) 15-HETE.IS	0.7806
16-HDoHE	343.0 / 233.0	16	(d8) 15-HETE.IS	3.3544
17-HDoHE	343.0 / 245.0	16.06	(d8) 15-HETE.IS	0.2779
20-HDoHE	343.0 / 241.0	15.72	(d6) 20-HETE.IS	1.3316
7R Maresin-1	359.0 / 177.0	11.33	(d4) LTB_4.IS	0.2837
PD_1	359.0 / 153.0	*	(d4) LTB_4.IS	No Primary
15t PD_1	359.0 / 153.0	*	(d4) LTB_4.IS	No Primary
10S,17S-DiHDoHE (PDX)	359.0 / 153.0	11.19	(d4) LTB_4.IS	1.0292
RvD_2	375.0 / 175.0	7.64	(d4) LTB_4.IS	0.3639
RvD_5	359.2 / 199.0	*	(d4) LTB_4.IS	No Primary
16,17-EpDPE	343.0 / 193.0	17.36	(d11) 14,15 DiHETrE.IS	0.2435
19,20-DiHDoPE	361.0 / 229.0	13.31	(d11) 14,15 DiHETrE.IS	1.1322
19,20-EpDPE	343.0 / 241.0	16.91	(d11) 14,15 DiHETrE.IS	2.2213
10-Nitrooleate	326.0 / 169.0	*	(d5) EPA.IS	No Primary
9-Nitrooleate	326.0 / 168.0	*	(d5) EPA.IS	No Primary

*Estimated of the retention times of oxylipins which did not have a primary standard are found in Appendix 1.3

Appendix 1.5 HPLC Solvent Gradient

Total Time (min)	Solvent A (%)	Solvent B (%)
0.0	100.0	0.0
0.5	100.0	0.0
2.0	75.0	25.0
9.0	55.0	45.0
10.0	40.0	60.0
14.0	25.0	75.0
14.5	10.0	90.0
15.0	0.0	100.0
17.0	0.0	100.0
19.0	100.0	0.0
35.0	100.0	0.0

Composition of solvent A and B is found in Appendix 2.4.3.5

Appendix 1.6. Internal Standard Used In Oxylipin Quantification

Internal Standard on Qtrap 6500			
Internal Standard	**Stock Volum(µL)**	**Stock Concentration (ng/µL)**	**Final Concentration (ng/µL)**
6k-PGF$_{1\alpha}$-d$_4$	30.0	25	0.75
TXB$_2$-d$_4$	20.0	25	0.50
PGF$_{2\alpha}$-d$_4$	20.0	50	1.00
PGE$_2$-d$_4$	10.0	50	0.50
PGD$_2$-d$_4$	50.0	25	1.25
13,14-dihydro-15-keto-PGF$_{2\alpha}$	20.0	50	1.00
LTB$_4$-d$_4$	80.0	25	2.00
20-HETE-d$_6$	80.0	25	2.00
15-HETE-d$_8$	40.0	25	1.00
5-HETE-d$_8$	80.0	25	2.00
13-HODE-d$_4$	40.0	25	1.00
9-HODE-d$_4$	40.0	25	1.00
12,13-DiHOME-d$_4$	20.0	25	0.50
9,10-DiHOME-d$_4$	20.0	25	0.50
14,15-DiHETrE-d$_{11}$	10.0	25	0.25
11,12- DiHETrE -d$_{11}$	10.0	25	0.25
8,9- DiHETrE -d$_{11}$	40.0	25	1.00
15deoxy-PGJ$_2$-d$_4$	80.0	25	2.00
EPA-d$_5$	40.0	50	2.00
5-OxoETE-d$_7$	270.0	25	6.75
Total	1000.0		

10µL was added to each sample (per 200µL kidney and liver homogenate and per 400µL serum) for oxylipin analysis.

Appendix 2 Protocols

Appendix 2.1 Protocol of Diet Preparation

All ingredients except cornstarch, dextrose, fiber (cellulose), and sucrose are found in the walk-in refrigerator. Labeled containers for weighing ingredients can be found in the refrigerator (these containers do not need to be washed after every use but must be kept in the refrigerator)

1. Measure out all ingredients according to recipe.
2. In a large bowl add sucrose and all ingredients weighing less than 200g (eg. Min mix, L-cysteine, methionine, etc). Mix by hand so all ingredients are thoroughly combined. This is an important step to ensure that smaller ingredients are well distributed in the diet.
3. In the mixing bowl (Hobart Mixer bowl) add half of all remaining ingredients (2.5kg) and half of the mixture of smaller ingredients. Mix thoroughly by hand and break up any large clumps. Add the remaining half of all ingredients and mix again by hand (2.5kg).
4. Attach Hobart Mixer bowl to the machine. Lock into place and raise the bowl up using the lever. Attach plastic shield.
5. Set mixing speed to 1 and timer to 3 minutes. Only turn the power switch on after timer has been set.
6. When mixing stops turn the power switch off, lower the bowl, and scrape the sides of bowl and the beater. Also, make sure to blend ingredients at the bottom of the bowl, as the beater cannot reach them. Once finished raise bowl back into place and attach the shield.
7. Set mixing speed to 2 and the timer to 5 minutes. Do not start the mixer yet. At this step you will need to add soybean oil.
8. Tare the scale with the container of soybean oil without the lid. Measure oils according to how much you need. Turn on the mixer and slowly begin to add the oil.

9. When mixing stops turn the power switch off, lower the bowl, and scrape the sides of bowl, the beater, and the bottom of the bowl again. Once finished raise bowl back into place and attach the shield.
10. Set mixing speed to 2 and the timer to 5 minutes again.
11. When mixing stops turn the power switch off, lower the bowl, and scrape the sides of bowl, the beater, and the bottom of the bowl again. Once finished raise bowl back into place and attach shield.
12. Set mixer speed to 2 and the timer to 5 minutes one last time.
13. Once mixing is finished remove the bowl from the machine and mix contents by hand and break up any remaining clumps.
14. The finished diet should be grainy in consistency.
15. Diet can now be placed in a large Ziploc bag and stored in the walk-in freezer.
16. Clean all equipment used. Wash down counters and machine. Sweep floor. Wash weight containers at the end of once every four diets are made.

Appendix 2.2 Protocol of Fatty Acid Analysis Of Oils

1. Weigh 0.11g of oil into a 13 X 100mm culture-tube. Add 1ml of hexane and vortex in fume hood. All solvent work should be done in the fume hood.
2. Add 10ul of internal standard (TG, C19:0)
3. Pipet 100ul of diluted oil (10mg) into an 8ml (13 X 100mm) screw top tube then evaporate solvent under nitrogen in a warm water bath at 30-35^{o}C.
4. Add 1ml of toluene to tube and vortex 5-10 seconds.
5. Add 1.2ml of methanolic HCl, cap tubs tightly with Teflon lined lids and vortex 5-10 seconds.
6. Place tubes in an 80^{o}C oven for 60 min.
7. Remove tubes from oven and allow to cool to room temperature (can put in cold water after 5-10 minutes if in a hurry.)
8. Add 1ml of distilled or deionized water and 1ml of hexane; cap and vortex for 20s.
9. Centrifuge at 800g for 5 min.
10. Transfer hexane upper layer to a clean 8ml screw top tube.
11. To the hexane layer add 2 ml of water; cap and vortex for 20 s.
12. Centrifuge for 5 min at 800g.
13. Transfer part of hexane layer to a GC vial (approx. ½ full); cap vial.

Appendix 2.3 Protocol of Lyophilisation

1. Fill Styrofoam container with ice.
2. Gather the amount of 15ml tubes required.
3. Poke 4 small holes in each of the lids
4. Label the tubes to correspond with the samples.
5. Remove 6 kidneys at a time from the -80° C freezer and put on ice.
6. Place beaker on balance and tare.
7. Weigh tube, including lid, recording all decimal places, now and throughout. Recording all decimal places becomes important as the kidney nears dryness.
8. Using a sterile blade, slice kidney into 3 or 4 sections, depending on fit, and place in corresponding tube. Screw on lid and record mass.
9. Place tube on ice.
10. Clean blade in 70% ethanol and wipe dry. Rinse blade in H_2O and wipe dry.
11. Repeat steps 6 to 9 with the remaining 5 samples.
12. Store samples in the -20° C freezer near the scale.
13. Remove 6 new samples from the -80° C and repeat same procedure for these samples.
14. Repeat steps 6 to 13 until all kidneys are weighed and stored in the -20° C freezer.
15. If the samples are not being lyophilized right away, store in the -80° C until they are ready to be freeze dried.
16. Please see Dennis regarding the use of the freeze dryer.
17. Samples will be put in the freeze dryer at the end of the day.
18. The next morning ask Dennis to remove the samples from the freeze dryer.
19. Keeping about 15 samples on ice, place the rest of the samples in the -20° C freezer near the scale.
20. Using a Kim wipe, ensure all ice and water has been removed from the outside of the tubes. Weigh the 15 samples, recording mass to the last decimal place, and place in the

-20° C freezer.

21. Take out 15 new samples and repeat the procedure.
22. Repeat steps 20 and 21 until all samples have been weighed.
23. Leave samples in the -20° C freezer for approximately 30 minutes before returning to the freeze dryer.
24. At the end of the day, repeat steps 19 to 23.
25. Repeat weighing the samples at time intervals until samples have the same mass as the previous reading. The mass may actually increase due to the dried kidney absorbing moisture from the atmosphere or leftover ice from the container.
26. The kidneys are now freeze dried. They should be transferred to labelled, small, disposable scintillation vials or 2.0ml microcentrifuge tubes.
27. To pulverize the sample, take an angled scoopula cleaned in 70% Ethanol and rinsed in purified H_2O. Scoopula should be wiped completely dry so as not to add water to the sample. Carefully use it to mash the kidney sample until no chunks remain in the tube.
28. When pulverizing, if static attracts sample to scoopula, wipe off sample into a folded, small wax weigh sheet where it can be transferred to the vial. Using a dryer sheet, wipe gloves on both hands and in between fingers. This should take care of the static.

Appendix 2.4 Protocol of Homogenization And Aliquoting Homogenate

Appendix 2.4.1 Protocol of Homogenization

Use Tyrode's (pH 7.6) salt solution to homogenize dried kidney tissue. For every 70 mg of dried (lyophilized) kidney tissue approximately 2000µL of Tyrode's (pH7.6) is required. Only homogenate kidney and liver need to be homogenized. Serum does not need to be homogenated. Therefore, 45 mg of dry (lyophilized) tissues = 1250 µL of Tyrode's required

* If using wet tissue, for every 350mg add 2000µL of Tyrode's (pH7.6). Similar ratios have been used for kidney and liver. For serum there is no need to homogenate

1. Ensure there is enough prepared of:
    - Tyrode's salt solution (pH 7.6) (Check for deterioration. (See Solutions Preparation below)
    - 1% Triton Solution as per instructions below (see Solutions Preparation).
    - 12 mL test tubes with lids that have been soaked overnight in Contrad solution, rinsed and dried.
    - 100:1 Methanol:Formic Acid
    - pH 3 water – (water that has had pH adjusted to 3.0 using 1M HCl)
    - Antioxidant Cocktail
2. Obtain a large container of ice
3. Remove the required lyophilized kidney samples from the -80C freezer and keep on ice.
4. Label 16 x 125mm disposable glass test tubes with sample ID's
5. If samples appear to be a fine powder proceed to weighing. If there are clumps in the sample, pulverize before weighing out sample.
6. Weigh and record 45 mg (can be +/- 2 mg – just make sure that the weight is recorded in your lab book) lyophilized kidney sample into labeled tubes (prepared in step 4), cover with

parafilm and immediately place on ice.

7. Return the remaining lyophilized kidney samples to the -80 freezer
8. Calculate, record, and add required amount of Tyrode's (pH7.6) to each massed kidney sample.

45 mg of tissues = 1250 µL of Tyrode's required

9. Prepare and label three disposable glass tubes (16 x 125mm) with 100% ethanol and three disposable glass tubes (16 x 125mm) with ultrapure water for cleaning the homogenizer
10. Clean homogenizer before use, after use, and in between each sample by:
    - 3 tubes ethanol x 30 seconds each at speed 15
    - 3 tubes ultrapure water x 30 seconds each at speed 15
    - dab with kimwipe to dry
11. Place test tube containing lyophilized kidney tissue in a small plastic container (yogurt container) containing an ice slurry (ice plus water).
12. Insert rotor into test tube and homogenize at speed 20 for 30 seconds. Avoid generating bubbles. Stop and check that all kidney tissue is at the bottom of the tube. If not, use rotor tip to push everything to the bottom of the test tube.
13. Homogenize again for another 30 seconds (speed 20).
14. Repeat steps 10-13 for each sample. Remember to keep tubes covered with parafilm and on ice as much as possible
15. When finished all samples, take apart rotor to wash thoroughly inside. When putting rotor back together do not tighten too firmly.
16. Any tools contaminated with biological hazards can be wiped off with 10% bleach then washed normally with Contrad / other detergent.

Appendix 2.4.2 Protocol of Aliquoting Homogenate

1. Fatty acids: Aliquot 250 µL into 12 mL tubes with screw top for fatty acid analysis (make sure these tubes have been soaked overnight in Contrad solution, rinsed and dried).

   *To clean tubes, prepare a 2-5% solution of Contrad (kept in cupboard under sink) and distilled water in a large beaker, submerge tubes and lids and let soak overnight. Once they are finished soaking thoroughly rinse with tap water, 3 to 5 times, followed by distilled water (3 to 5 times) and let dry upside-down on a rack. Immediately add 8.34 µL of the antioxidant cocktail to the Fatty Acid Analysis tubes (6.67µL antioxidant for every 200µL of homogenate) and put back on ice if proceeding directly to fatty acid analysis. Otherwise, flush with nitrogen, cap and store at -80°C.

2. Oxylipins: Remove an aliquot (200µL for each tube, total 2 tubes) for the oxylipin analysis and place in a microcentrifuge tube for liver and kidney; 400µL serum is used directly.

   1) Add 1µL of a 1% Triton solution for every 100 µL aliquot for oxylipin analysis. (Final concentration of Triton in homogenate should be 0.01%: 0.01x 100=1µl)
   2) Vortex for 10 seconds
   3) Incubate, covered, on ice for 10 minutes
   4) Vortex again for 10 seconds
   5) Incubate, covered, on ice for 10 minutes
   6) Vortex again for 10 seconds
   7) Incubate, covered, on ice for 10 minutes
   8) Vortex and aliquot 200 µL of tyrode's homogenate (this represents 30mg of wet tissue) into two labeled 2 mL microtubes (if doing duplicates).
   9) Vortex each 2 mL microtube for 10 seconds.
   10) Working quickly, add in the same order below to the samples;
      a) 500 µLof 100:1methanol formic acid
      b) 800 µLof pH3 water
      c) 90 µL of 100% ethanol

d) 10 uLof antioxidant cocktail

e) Vortex for 5 seconds.

11) Store samples in -80C freezer for future oxylipin extraction – see Appendix 2.5.

Appendix 2.4.3 Protocol of Solutions Preparation

Appendix 2.4.3.1 Protocol of Reconstitute Tyrode's Salts Without Sodium Bicarbonate

Product# T2145 (Sigma)

Tyrode's salts powder comes prepackaged from Sigma-Aldrich and is kept in the fridge. Please refer to the product insert for full product information. Powdered salts are hygroscopic and should be protected from moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated salt solution is not recommended as precipitates may form. Tyrode's Salts are meant to maintain cellular pH and osmotic balance.

1. Measure out 1L of ultrapure water in a plastic graduated cylinder. Water temperature should be 15-20C
2. Transfer about 800 mL of measured water into a 2000 mL flask. Add a large stir bar (careful not to splash) and place on stir plate. Begin gentle stirring.
3. Add powdered Tyrode's salts and continue stirring until dissolved. Do NOT heat.
4. Rinse original Tyrode's salts packaging with some (NOT all) of the remaining 200 mL measured water to remove all traces of powder. Add to solution in step 3. Rinse the package 3 times.
5. Transfer solution to a 1L volumetric flask and bring to volume using some of the remaining 200 mL measured water in graduated cylinder from step 1. Rinse the flask 3 times. Can use a pipette to bring the flask up to volume w/ water.
6. Insert volumetric stopper and invert 10x to mix. Before inverting, ensure the stopper and mouth of the volumetric flask are completely dry to prevent leaks.
7. Transfer to a 1L glass bottle covered with tin foil to protect from light and clearly label as Tyrode's salts without $NaHCO_3$. Store in the refrigerator (2-8C).

Appendix 2.4.3.2 Protocol of To Make 100mL of Tyrode's (pH 7.6)

1. Measure 100 mL of reconstituted Tyrode's salt solution into a graduated cylinder.
2. Weigh 100mg of powdered sodium bicarbonate (Sigma, S5761) into a 125 mL Erlenmeyer flask.
3. Cover flask with tin foil to protect from light. Add a stir bar.
4. Transfer about 80 mL of measured Tyrode's in graduated cylinder from step 1 to the Erlenmeyer flask.
5. Completely dissolve powder into solution by placing on stir plate and stirring (apprx 15 min). Powder must be completely dissolved before adjusting pH.
6. While continuing to stir, adjust the pH of the solution to pH 7.6 using 1N HCl or 1N NaOH. Normally to achieve pH 7.6, a couple of drops of 1N HCl are required.
7. Transfer solution to a 100mL volumetric flask and bring to volume using some of the remaining 20 mL measured Tyrode's in graduated cylinder from step 1.
8. Insert volumetric stopper and invert 10x to mix.
9. Transfer to a 100mL glass bottle covered with tin foil to protect from light and clearly label as Tyrode's salts (pH 7.6). Store in the refrigerator (2-8C).

NOTE: Tyrode's that has sodium bicarbonate added and has been pHed can deteriorate.

Deterioration can be recognized by:

- pH change
- precipitate or particulates
- cloudy appearance
- colour change

Check all these signs before using.

Appendix 2.4.3.3 Protocol of Making 1% Triton By Using Tyrode's (pH 7.6)

This solution mixes best if Tyrode's (pH 7.6) is at room temperature.

1. Weigh out 0.02 g of Triton solution in a 20 mL scintillation vial
2. Add 2.0 mL of room temperature Tyrode's (pH 7.6) using a 1.0 mL eppendorf pipette.
3. Cover with cap and vortex well.
4. Cover with tin foil to protect from light and chill on ice or in fridge. Store remains in refrigerator.

A final concentration of 1% Triton is required in the homogenate to disrupt lipids and release proteins. This will ONLY be added to the LC-MS/MS fraction.

Therefore add 10μL of 1% Triton (pH 7.6) to 1000μL of kidney homogenate for the LC-MS/MS fraction only to make a 0.01% Triton final solution

i.e. Volume 1% Triton to add (μL) = (0.01 final concentration)(1000μL kidney homogenate)

Appendix 2.4.3.4 Protocol of Making Antioxidant Cocktail

Content: 0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2mg/mL TPP, 2 mg/mL Indomethacin in a solution of 2:1:1 MeOH:EtOH:H_2O.

Make a minimum of 100 mL Antioxidant Cocktail. Measure out 50 mL of Methanol and 25ml Ethanol in separate graduated cylinders. Mix together in a 250 mL beaker. Cover the outside of the beaker with tinfoil and place on magnetic stirrer. Put stir bar in beaker and cover the top with tinfoil to minimize volatilization. Weigh out 20 mg BHT, 20 mg EDTA, 200 mg TPP and 200 mg Indomethacin onto separate weigh paper. Add the ingredients to the MeOH:EtOH solution and stir solution until all dissolved. This will take a while. Keep beaker completely covered with tinfoil to also minimize exposure to light. When all dry ingredients are dissolved, transfer the mixture to a 100ml volumetric flask. Using a small amount of ddH_2O, wash down the sides of the beaker and pour into the 100ml flask. Do this 3 times to ensure you transfer all of the solvent and antioxidants. Fill up the 100ml volumetric flask to the mark with ddH_2O. Stopper flask and invert 10x to mix. Transfer into a clean, tinfoil covered, labeled 125 mL bottle. Aliquot the appropriate amount of antioxidant cocktail into covered scintillation vials for individual users.

Appendix 2.4.3.5 Protocol of Making Solvent A and Solvent B

Solvent A

Water – Acetonitrile – Acetic Acid [70:30:0.02; v/v/v]

*MS Grade

*Prevent evaporation of prepared solutions using paraffin around cap seals

To make 1000 mL:

700 mL water

300 mL acetonitrile

200 µL acetic acid

Vacuum filter through Whatman #4 filter paper

Solvent B

Acetonitrile – Isopropyl Alcohol [50:50; v/v]

*MS Grade

To make 1000mL:

500 mL Acetonitrile

500 mL Isopropyl alcohol

Vacuum filter through Whatman #4 filter paper

Appendix 2.5 Protocol of Solid Phase Extraction For Oxylipins

1. Get ice and pull out kidney and liver samples or serum and directly to thaw on ice.
2. Turn on centrifuge and cool to 4°C.
3. Turn on nitrogen evaporator both to 37°C.
4. Add 1mL of pH3 water into a 2.0mL microtube; add 750µL kidney and liver aliquote from protocol 4 and 400µL of serum sample. Vortex.
5. Add 10µL of internal standard to each tube. Vortex. Run nitrogen over internal standard before capping and storing it in the -20°C freezer.
6. Vortex then test pH of mixture using 5ul of sample spotted on appropriate pH strips. If necessary acidify to pH3 with 10M HCl, usually around 4.5µL. Vortex before testing again.
7. Centrifuge test tubes for 5mins @ 3000rpm & 4°C to remove debris.
8. Set up enough columns for each sample in the fume hood. Use the Strata-X 33u Polymeric Reversed Phase Columns (60mg/3mL) (Phenomenex).
9. Place a waste vial under each column.
10. Pre-condition the column with 1ml methanol. Allow the methanol to drip through for 1 minute, then gently apply pressure with a BD 10ml syringe to increase flow. Make each drop dropping down around 1.5-2 seconds apart. Do not allow column to go dry.
11. Add 2000µL of pH3 water to each column. Push through in the same way.
12. Using a "kimwipe collar" to avoid water from the outside of the tube to drip, apply sample to column. Let it drip through by gravity.
13. Add back 1000µL of 10% methanol in pH3 water to each sample test tube. Vortex and centrifuge the same way as in steps 7 and 8.
14. While centrifuging, label the needed amount of 1.5mL microtubes.

15. Push through sample in columns with gentle pressure now if necessary; then apply the new mixture from step 14 to the columns. Push this through to dry using 2 BD 10ml syringe fulls of air. Make each drop dropping down around 1.5-2 seconds apart.
16. Set up microtubes underneath the columns.
17. Add 1000µL of 100% methanol to each column. Allow dripping through for at least 1 min, and then push through with gentle pressure. Make each drop dropping down around 1.5-2 seconds apart. Run column dry by pushing through with syringe a few (3-5) times.
18. If samples not being run today, cap with nitrogen and store at -80°C (dry down later).
19. Ensure the nitrogen evaporator is at 37°C and clean needles with 100% chloroform.
20. Set up microtubes in the evaporator and dry down the samples, checking on them every 15 min.
21. Once samples have dried down completely, add 100µL of solvent A to each of the microtubes. Run nitrogen over tubes before capping and then vortex.
22. Samples can be stored in the -20°C freezer at this point if needed for up to 1 week.
23. Label GC/LC vials and add a 200µL target propylene conical insert into each one.
24. Ensuring samples are thawed, vortex them.
25. Centrifuge samples at 4°C for 1 min @ 7000rpm.
26. Transfer each sample into its appropriate via and store in the -20°C freezer and run them within 1 week.

Appendix 2.6 Protocol of Analyzing LC-MS Data Using Multi-Quant

General Rules and Tips for Selecting Peaks

1. Click on small icon with the graphs to view the data in graph form. Click on the magnifying glass icon to zoom in.
2. Now the data is set up to go back and forth between the table and the graphs.
3. The Internal standards are listed first. These usually have the correct peak selected but it is a good idea to go through all of them to make sure.
4. After the IS list all analytes are listed and shows all samples for that one analyte in the graphs.
5. If the specific analyte has a deuterated internal standard select the peak according to the peak in the internal standard.
6. If the analyte does not have a deuterated internal standard look at the printed primary list. If there is a primary for that analyte look at the primary's graph and the peak will tell you the retention time for the samples.
7. If there is no primary for the analyte, the next table to look at is the one that lists all analytes with their expected retention times. These retention times are just approximate to your samples and your samples will generally come slightly after these.
8. The samples are in order that they run in (index number). If not, sort them in order of index number. It is expected that there will be some drift of the retention time as you go through the samples.
9. The peaks should be >5x the baseline. If not, click "select peak to not found icon" at the top.
10. The peaks should also include an area $\geqslant$ 3.000e3. If not, click the "select peak to not found icon."
11. Some peaks will have a "shoulder" or two peaks in one. To view them as 2 separate peaks in the space for "Gaussian Smooth Width" change the 2 to 1 and "Apply." You should then be able to select the correct peak.

12. Sometimes it is hard to determine the baseline. To zoom in on a graph, click and drag along the axis of the graph to where you want to zoom. If you want to zoom in on all graphs right click on the graphs and select “Options”, go into the tab called “Zooming” and under “zooming access intensity” select 1000cps. This will zoom in all of the samples but make sure to put it back to original setting (100 percent of largest peak) after looking at the samples.
13. Don’t be misled by very small peaks. If the baseline is very low the peak could still be 5x above the baseline. Zoom in for a closer look to determine this.

Appendix 2.7 Protocol of Fatty Acid Analysis

Appendix 2.7.1 Protocol of Fatty Acid Extraction

1. Transfer 250ul of tissue homogenate or serum into a 12ml glass tube and prepare a blank tube with 250µL of Tyrode's with every set of samples you do. We can work with 12 samples at a time including the blank.
2. Note that all work with solvent must be done in the fume hood. Add 2.5mL (2500ul) 2:1 chloroform:methanol with 0.01% BHT to your homogenate and blank. To prepare solution: 0.03g butylated hydroxytoluene, 200mL chloroform, 100mL methanol = 0.06g BHT, 400mL chloroform, 200mL methanol. Vortex.
3. Add 10µL of C15:0 standard and 10µL of C17:0 mix using a 100µL pipette.

   Standards:

   C15:0 Phospholipid (10mg/ml) – add 10µL for Phospholipid (PL) analysis

   C17:0 Free Fatty Acid (2mg/ml) and C17:0 Triglyceride (5.5mg/ml) 1:1(mass:mass) mixture– add 10µL for other fatty acids which excludes phospholipid analysis
4. Add 2.25mL (2250ul) of 2:1 chloroform:methanol to the homogenate.
5. Cap and vortex for 15 seconds.
6. Add 950ul 0.73% NaCl. To prepare solution: 0.73g NaCl in 100mL de-ionized water = 3.65g NaCl in 500mL de-ionized water.
7. Cap and vortex for 30 seconds, put on ice.
8. Centrifuge for 10 minutes at 800g. It is crucial to place and balance tubes properly or breakage may occur. If you have an odd number of tubes, add an extra tube with similar solvents and water volumes to balance the load.
9. Get second set of 12mL glass vials with Teflon lids and label with sample ID's using tape.
10. Take the tubes out of the centrifuge when completed. You should see 2 layers. Using a glass Pasteur pipette with small yellow bulb, carefully extract the lower phase without taking in

the upper phase. Slide the tip of the Pasteur pipette down the side while pushing out bubbles, and slowly draw up the lower phase and release it into your newly labeled 12ml vials. If the 2 layers were accidently disturbed and mixed, re-centrifuge the tube.

11. Before placing your samples in the nitrogen bath, turn on the nitrogen tank so that a gentle stream comes out the open the needles that you will be using. Wash the needles with chloroform (place the needle in a scintillation vial with 100% chloroform for 5 seconds) and then let any remaining chloroform volatilize.
12. Place your samples (in the 4mL vials) in the nitrogen bath with the lids off. Carefully lower the needles close to the sample and turn the nitrogen gas on until you see a gentle ripple in the sample. Keep in the nitrogen bath until the vial is completely dry.
13. Remove samples from nitrogen one at a time adding 100µL of 2:1 chloroform:methanol . Blow nitrogen over sample, cap and vortex. Samples are vulnerable to oxidation between being removed from nitrogen and adding solvent.
14. If temporarily stopping at this point, store 4mL vials in -20C freezer to continue later.

Appendix 2.7.2 Protocol of Thin Layer Chromatography (TLC)

1. Turn on oven to around 88°C (or lower heat if still on from heating TLC plate). The temperature desired for methylation is 80°C, but when the oven is opened, the temperature rapidly drops. 8-10°C above desired temperature gives some leeway.
2. Make up the solvent tank and let it equilibrate for at least half an hour.
    - Cut a piece of chromatography paper to fit in tank so that it covers the back and sides. You should only need to fold the paper in half lengthwise and tear.
    - To make solvent for tank, get a glass graduated cylinder (100ml or 250ml), in fume hood fill graduated cylinder with 60ml of Heptane, 40ml of Isopropyl ether, and 3ml of acetic acid. You can pour heptane and isopropyl ether into graduate cylinder so it reaches 100ml then pipette 3ml of acetic acid in. Pour solvent mixture into tank and close, fastening on lid. Do this in the fume hood and leave tank in the fume hood for solvent to volatilize.
    - Depending on which solvent method is appropriate, you may be using 85.0ml petroleum ether, 15.0ml diethyl ether and 0.5ml acetic acid in the chromatography tank.
3. Using the TLC template with 6 or 8 lanes (depending on how many samples you have), create equal and parallel vertical lanes on your silica plate (that was previously heated) using the dull side of a razor blade. Be careful not to make any marks on any other part of the plate or to touch the plate (you could accidentally transfer lipids onto the plate from your gloves). Leave at least 1cm on each side of plate. Only use pencil on the plate, no ink.
4. Turn on nitrogen tank with small drying hose attached. Using a 100ul pipette, draw up 50µL of your sample and slowly spot it along the horizontal line drop by drop in the center of the lane. After each drop, dry with nitrogen hose.
5. Place the plate in the tank until the solvent line reaches 1 cm from the top. Keep plate as vertical as possible. This will take approximately 20-30 minutes. Make sure to check the solvent front at 20min. While plate is running, label your 12ml tubes with sample ID and either PL, TG, Chol, FFA and get your weigh paper ready for scraping plate (fold creases)

6. Let solvent volatilize off TLC plate in the fume hood. You'll know it has when the plate is removed from the fume hood and you don't smell a strong odor of *solvent* (a slight odour of acetic acid, ie vinegar, is fine). Dry the plate with a gentle stream of nitrogen gas. Samples are again susceptible to oxidation, so don't dry for any longer than 10 minutes. It should only take a minute or two using nitrogen gas to dry.
7. Protect the back of the fume hood and other samples in the fume hood by placing a large piece of cardboard along the back of the fume hood. Stand up TLC plate in the center of the cardboard.
8. Attach hose to the nitrogen gas in fume hood and to bottle with 0.1 % ANS solution in water and spray a fine layer onto the TLC plate. 0.1% ANS can last about a week. Check the color of the solution; if it is a dark yellowish/brown, it is no longer good to use. To prepare 0.1% ANS, weigh out 100 mg ANS (8-Anilino-1-Naphthalene-Sulfonic Acid) and add 100 mL of milli-Q water and stir on stir plate for about 30 minutes. Protect from light using tinfoil.
9. In a dark room wearing UV safety glasses, use the hand held UV light to mark the PL, FFA, Chol and TG lines using a pencil, indicating where to scrape. R_f (retention factor) for PL = 0, R_f for TG =0.61 (PL is at the origin and TG is about 60% of the way to the solvent front).
10. Since static is a factor, wipe your gloves with a dryer sheet and keep it handy for further use if needed. Be careful not to contaminate your samples with the dryer sheet.
11. Wear a mask when scraping as silica dust is extremely hazardous. Also, work in an area of the lab away from others. Using a razor blade, scrape the indicated portions onto a creased weigh paper and carefully transfer to the corresponding, labeled 12mL screw top test tube.
12. Add 1.2mL (1200ul) of methanolic HCl (or methanolic sulfuric acid) kept in fume hood. A solution of 6% methanol sulfuric acid is made by slowly adding 6ml of sulfuric acid to 94ml of methanol and can only be used fresh (up to 1 week). Use Teflon tape, cap tightly and vortex. If stopping here, store overnight in -20C. Note methanolic sulphuric acid is only good for about 1 week. Then dispose of it in appropriate waste bottle.

Appendix 2.7.3 Protocol of Fatty acid Methylation

Turn on oven (about 88°C for heat up and temperature will drop 8-10°C when you open the door) and allow it to reach temperature, if it is not already on

1. With a marker, mark the liquid level of each tube. Place tubes in a metal rack in a preheated 80° C oven for 1.5 hours. After 30 minutes if the volume decreases, remove the tubes from the oven, let cool, add more methanolic acid to achieve the original volume and place back in oven
2. After 1 hour, remove rack from oven and cool for 10-15 minutes (if the liquid is leaking, then extend it to 1.5 hours).
3. In the fume hood, add 1.5mL (1500ul) toluene to the tubes, cap tightly and vortex for 30 seconds.
4. Add 1mL of ultrapure water to the tubes, cap and vortex for 15 seconds. Centrifuge for 5 minutes at 800g.
5. Using glass Pasteur pipette tip with yellow rubber bulb, transfer the top layer to a clean 2mL GC vial (glass). Place in the -20°C freezer.
6. Once the solvent has evaporated, immediately add 200µL of hexane to the tubes and cap. Store the samples in the -20°C freezer in a labeled box. When they are ready to be run on the GC, label GC vials with sample number, date and your initials and transfer 100µL into a glass insert with spring. Cap and store in -20°C freezer in a sample box clearing stating that they are ready to be run on GC and marked to place an analytical service request.