ALA and DHA Rich Oils Alter Blood Oxylipin Profiles Differently in Young Healthy Males and Females

by

Melissa Gabbs

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Human Nutritional Sciences

University of Manitoba

Winnipeg, Manitoba, Canada

R3T 2N2

I. ABSTRACT

Time course changes in oxylipin profiles among healthy, young individuals consuming high doses of alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) remain to be determined. Differences in lipid metabolism suggest the importance of separating sexes when investigating the effect of omega-3 supplementation on the oxylipin profile. Individuals (n=12) participated in a double-blind randomized cross-over trial where ALA oil (4g/day ALA) and DHA oil (4g/day DHA) were consumed for four weeks. Oxylipins from plasma, serum, and supplemental oils were analyzed. Females responded more immediately than males to DHA oil treatment and had higher levels of several DHA derived oxylipins, while ALA oil had a minimal effect on oxylipin production. Several oxylipins were elevated in serum when compared to plasma. Further, oxylipins were present in both supplemental oils. These results can be used to further explore oxylipin profiles in males and females and to help explain the impact of omega-3 supplementation.

.

Acknowledgements

I would first like to thank my supervisor, Dr. Harold Aukema for his endless guidance and support throughout the entirety this project. I would also like to thank Dr. Peter Zahradka and Dr. Carla Taylor for their help and support, especially during the clinical phase of my work.

I would secondly like to thank my lab including – Tanja, Dennis, Dennis, Chelsea, Jessay, Shan, Monir, Lucien, Anne, Arif, Afroza, Nikhil, and Sam for their technical support and assistance. I could not have succeeded thus far with without their willingness to help and listen. I would also like to thank the clinical staff at the Canadian Center for Agri-Food Research in Health and Medicine including Danielle, Angela, and Terri. They truly made the last two years extremely enjoyable and I will miss having such wonderful colleagues. I additionally would like to thank Dr. Depeng Jiang for his assistance with the statistical analysis for this study. I would also like to thank the individuals who participated in this study, as for without them I would not have a study at all.

I would like to thank the Canadian Institutes of Health Research, Government of Manitoba, and the Faculty of Graduate Studies for their financial support. Also, the Canadian Center for Agri-Food Research in Health and Medicine and the Asper Clinical Research Institute at St. Boniface Hospital for use of their facilities.

Lastly, I would like to thank my family, friends, and Anthony for their love, support, and patience throughout my entire education. Thank you for providing balance in my life.

II. TABLE OF CONTENTS

III. LIST OF TABLES

IV. LIST OF FIGURES

V. LIST OF ABBREVIATIONS

ALA, α-linolenic acid; AA, arachidonic acid; ASA, acetylsalicylic acid; BHT, butylated hydroxytoluene; BMI, body mass index; BP, blood pressure; cAMP, cyclic adenosine monophosphate; CID, collision-induced dissociation; COX, cyclooxygenase; cP450, cytochrome P450; CVD, cardiovascular disease; DGLA, dihomo-gamma-linolenic acid; dh, dehydro; DHA, docosahexaenoic acid; DiHDPE, dihydroxy-docosapentaenoic acid; DiHEPE, dihydroxy-eicosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHODE, dihydroxy-octadecadienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHOTrE, dihydroxy-octadecatrienoic acid; DPA, docosapentaenoic acid; EDTA, ethylenediamine tetraacetic acid; EPA, eicosapentaenoic acid; EpDPE, epoxy-docosapentaenoic acid; EpETE, epoxy-eicosatetraenoic acid; EpETrE, epoxy-eicosatetraenoic acid; EpODE, epoxy-octadecadienoic acid; EpOME, epoxy-octadecamonoenoic acid; FLAP, 5-Lipoxygenase activating protein; GC, gas chromatography; GCPR, G-coupled protein receptor; GLA, gamma-linolenic acid; HDL, high density lipoprotein; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; HpDoHE, hydroperoxy-docosapentaenoic acid; HpEPE, hydroperoxy-eicosapentaenoic acid; HpETE, hydroperoxy-eicosatetraenoic acid; HPLC-MS/MS, high performance liquid chromatography tandem mass spectrometry; HpODE, hydroperoxy-octadecadienoic acid; HpOTrE, hydroperoxy-octadecatrienoic acid; HX, hepoxilin; k, keto; LA, linoleic acid; LDL, low density lipoprotein; LiHEP, lithium heparin; LOX, lipoxygenase; Mar, Maresin; MUFA, monounsaturated fatty acid; LT, leukotriene; LX, lipoxin; NPD, neuroprotectin; NSAID, non-steroidal anti-inflammatory

drug; oxoEDE, oxo-eicosadienoic acid; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; oxoOTrE, oxo-octadecatrienoic acid; PD, protectin; PG, prostaglandin; PMN, polymorphonuclear; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; Rv, Resolvin; sEH, soluble epoxide hydrolase; SEM, standard error; SST, serum separator tube; t, trans; TC, total cholesterol; TPP, triphenylphosphine; triHOME, trihydroxy-octadecenoic acid; TrX, trioxilin; TX, thromboxane

1. CHAPTER 1 – GENERAL INTRODUCTION

Introduction

Oxylipins are oxygenated metabolites of polyunsaturated fatty acids (PUFA) that have numerous functions including roles in homeostasis and inflammation. Omega-3 fatty acids, including ALA and DHA are important in the diet and they are metabolized into oxylipins called octadecanoids and docosanoids, respectively. Due to more precise lipidomic analyses utilizing high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), analysis of the full range of oxylipins is now possible. This is critical for ALA- and DHA-derived oxylipins because they have been studied far less than those originating from twenty carbon fatty acids (eicosanoids). Omega-3 PUFA supplementation impacts the oxylipin profile through several variables including sex, time course changes, and supplement dose and composition. How these differences in lipid metabolism affect changes in oxylipin profiles between males and females during periods of supplementation remains to be determined.

1.1 Fatty Acids

Fatty acids are hydrocarbons with a carboxylic acid group at one end and a methyl group at the other. These hydrocarbons are hydrophobic in nature and can vary in length. They can be saturated, containing no double bonds, or unsaturated, containing one or more double bonds within their structure. Unsaturated fatty acids are classified based on the placement of their last double bond from the methyl group and can be omega-3 (ω3), omega-6 (ω6), or omega-9 (ω9) for example. Fatty acids with one double bond are said to be monounsaturated fatty acids (MUFA) whereas fatty acids with more than one double bond are referred to as PUFA. A number of PUFAs are essential in the diet and they play very important roles within the body.

Fatty acids are acquired in the body via two main ways: ingestion and conversion. When ingested, they are absorbed into the body via the enterocyte. Once absorbed, fatty acids can be utilized in several ways including conversion to other fatty acids, oxidation, storage, or incorporation into membranes as components of phospholipids [1]. In order to reach their target tissues these hydrophobic fatty acids are transported through the lymphatic system and the blood stream either as a part of a lipoprotein or complexed with albumin.

Fatty acids can be maintained in their free form in the body, however, they are more commonly integrated into a number of different molecular structures including triacylglycerides, phospholipids, and cholesterol esters. Triacylglycerides are a storage form of lipid where three fatty acids are attached to a glycerol backbone. Phospholipids are polar molecules that are made up of two fatty acids and a phosphate group attached to a glycerol backbone. Finally, cholesterol esters are cholesterol molecules esterified to a fatty acid. Phospholipids make up the bulk of the lipid bilayer of eukaryotic cells and they function as a semipermeable membrane, disallowing most particles from entering or exiting the cell freely. Although they can exist in their free form, most fatty acids exist as part of a structure and are only released from triacylglycerides, phospholipids, or cholesterol esters if needed as metabolic substrates or for transport into or out of cells.

Fatty acids are incorporated into the membrane phospholipids as part of a dynamic cycle of synthesis and degradation. These fatty acids can then be mobilized in response to cell activation and are either re-esterified into the cell membrane or oxygenated enzymatically or non-enzymatically to act as lipid mediators [2]. Further, dietary fatty acids can be incorporated into cell membrane phospholipids, altering membrane composition [3-5].

Essential Fatty Acids

There are two fatty acids essential in the diet of humans; these are linoleic acid (LA) and ALA, omega-6 and omega-3 fatty acids, respectively. LA and ALA are required in the diet as they cannot be synthesized endogenously and are important precursors for longer-chain fatty acids. Both LA and ALA are converted to longer-chain fatty acids via several enzymatic steps, and this process occurs for the most part in the hepatocyte endoplasmic reticulum as well as in peroxisomes [2]. Due to the differences in double bond placement on the omega-6 versus omega-3 fatty acids, they appear to perform different functions in the body.

In recent years there has been much debate about the dietary recommendations for LA, ALA and their long chain metabolites as researchers are seeking to determine recommendations that not only minimize deficiency risk, but also protect against cardiovascular disease (CVD) and other chronic illnesses within the population. A number of studies have looked at the roles that omega-6 and omega-3 fatty acids play in various disease conditions and the results seem to be varied. In 2009 the American Heart Association released a position statement that encouraged omega-6 consumption to prevent heart disease [6], however, the meta-analyses conducted recently by Chowdry *et al.* found no evidence to support this statement [7], leaving many questions unanswered regarding the consumption of omega-6 fatty acids. Further, negative effects in response to high omega-6 PUFA consumption have been reported including cardiac lipotoxicity and intestinal inflammation in mouse models [8, 9]. Finally, in a male population, replacing saturated fat in the diet with LA caused increases in death from all causes as well as higher rates of CVD [10].

Many studies have examined the role of omega-3 fatty acids in the body, particularly those derived from fish oils, and these meta-analyses have suggested that consumption of omega-3 long chain PUFAs may lead to a reduced risk of CVD [11, 12], as well as reduce the risk of

cardiac death [13]. Fleming and Kris-Etherton conclude that ALA may also have a beneficial role in lowering CVD risk, however, they suggest more well-designed studies need to be conducted to determine the optimal recommended amount [14]. Much is still unclear regarding omega-6 and omega-3 PUFAs and more research on their endogenous effects is needed to help identify their function in healthy humans as well as their link to CVD and other disease conditions. The precise mechanisms by which essential fatty acids affect human physiology are not currently clear. Examination of their oxygenated metabolites may shed light on their beneficial and detrimental effects, especially given the abundance of these metabolites in the body and the potent bioactivities described for the few that are previously studied.

Linoleic Acid

LA (C18:2ω6) is an omega-6 PUFA and is important in the diet because of its conversion to longer-chain fatty acids, namely arachidonic acid (AA,C20:4ω6). LA is found in many different foods in the North American diet and it is the main fatty acid found in vegetable oils. The Institute of Medicine has set the Adequate Intake for LA to be 17 g/d for males and 12 g/d for females [15], while the average intake for American males is 12-17 g/day and 9-11 g/day for females [16]. Conversion of LA to AA is important in the body because AA is one of the precursors for novel oxygenated metabolites having a variety of different metabolic and cellular functions.

LA is converted to AA via several enzymatic steps that include the desaturase and elongase enzymes. The first step of the pathway converts LA to gamma-linoleic acid (GLA) via delta 6 desaturase, followed by conversion into dihomo-gamma-linoleic acid (DGLA) by elongase, and finally conversion into AA [17]. While the conversion rate of LA to AA is unknown, it is thought to be efficient [2]. Refer to **Figure 1-1** for a graphical representation of

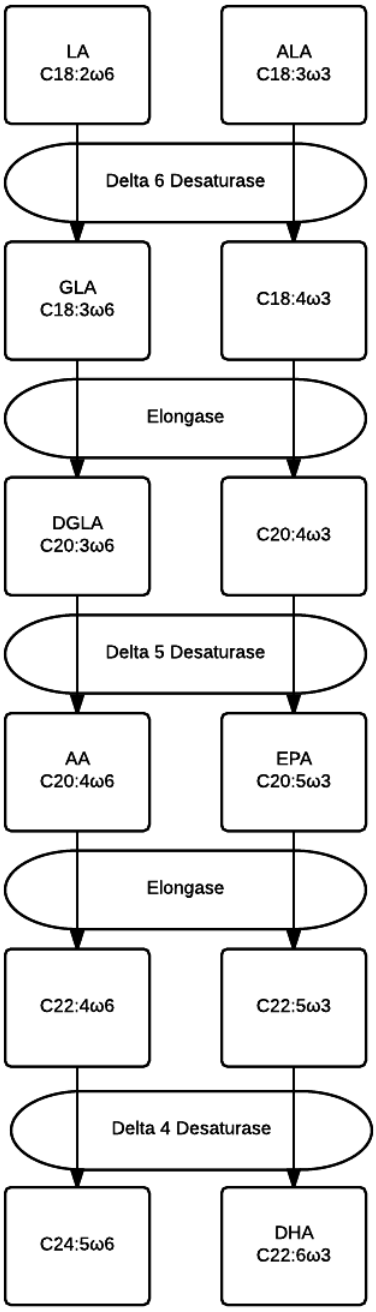


Figure 1-1. Conversion of omega-6 and omega-3 fatty acids in humans [18].
Linoleic acid and α-linolenic acid are converted via several enzymatic steps to long chain metabolites.

this pathway. AA can be further converted to adrenic acid (C22:4ω6), a fatty acid that is found in the adrenal glands, kidneys, brain, and the vasculature [19]. Adrenic acid is also a PUFA and can be converted into oxygenated metabolites.

Alpha-Linolenic Acid

ALA (C18:3ω3) is an omega-3 PUFA and is the precursor for very long-chain omega-3 fatty acids including eicosapentaenoic acid (EPA) and DHA. Similar to LA, ALA cannot be endogenously produced and therefore must be obtained through the diet in sources such as flaxseed products and walnuts. The Institute of Medicine has set the Adequate Intake for ALA, based on the actual average intakes of Americans, to be 1.1 g/day for females and 1.6 g/day for males [15]. However, an analysis of the National Health and Nutrition Examination Survey done in 2014 revealed that approximately 41% of American adults were consuming less than the Adequate Intake recommendations [20].

ALA is also particularly important in the human body because of its ability to be converted into EPA (C20:5ω3), which can be further converted into DHA (C22:6ω3). EPA and DHA can also be consumed preformed through the diet in various types of fish and marine oil products. Although conversion from ALA is suspected to be low [21], EPA and DHA are produced endogenously via desaturase and elongase enzymes similar to the conversion of LA to AA (**Figure 1-1**). To produce EPA, ALA is converted to C18:4ω3 by delta-6 desaturase, which is further converted to C20:4ω3 via elongase. C20:4ω3 is then converted to EPA by delta-5 desaturase [21]. EPA, via elongase, is converted to docosapentaenoic acid (DPA, C22:5ω3) and finally, DPAω3 is converted to DHA via delta-4 desaturase [18]. While the rate of conversion from ALA to DHA is estimated to be only 1% [22], it has been shown that young females may have a greater synthesis of EPA and DHA compared to males [23].

DHA and EPA in marine omega-3 oil have several effects, such as decreasing triacylglycerides (TG), very low-density lipoprotein-cholesterol, very low-density lipoprotein - TG, and slightly increasing LDL in patients with type two diabetes [24]. Further, EPA and DHA down regulate sterol regulatory element binding protein 1c, a transcription factor involved with lipogenesis [25]. DHA plays many additional roles in the body. For examine, it has been shown to decrease markers of inflammation and reduce circulating white blood cells and C-reactive protein compared to an olive oil placebo [26], and DHA but not EPA was evidently more effective at lowering blood pressure in overweight men [27].

As was shown in **Figure 1-1**, omega-6 and omgea-3 fatty acids utilize the same enzymes for the elongation and desaturation of their carbon chains, and at these steps is where competition between fatty acids is likely to occur. The amount of available substrate can alter the products of this conversion process and high levels of omega-6 fatty acid in the diet can lead to decreases in omega-3 fatty acid conversion due to competition for these enzymes [14]. This is the basis for examining the importance of the omega-6/omega-3 ratio and why it may be important to ensure adequate amounts of omega-3 fatty acids in the diet.

Sex Differences in Human Fatty Acid Profiles

In addition to the previously mentioned differences that exist between males and females regarding conversion to downstream metabolites, additional factors can influence the fatty acid profiles of humans. There are also sex differences in the metabolism of particular fatty acids, including ALA, which may result in: decreased β-oxidation rates in females as well as possible increases in fatty acids available for conversion and storage tissue fatty acid composition in females, as reviewed by Burdge and Calder [21].

Further, in a study examining adipose tissue fatty acid profiles it was found that women displayed lower adipose saturated fatty acids and higher monounsaturated and PUFA than their male counterparts [28]. Specifically females had higher adipose DHA and LA but lower AA [28]. Furthermore, aging is associated with a more profound change in adipose fatty acid composition in women compared to men. Older women (50-59), who may be going through menopause have lower PUFA, higher monounsaturated fatty acids and lower saturated fatty acids than younger or pre-menopausal women [28]. In men, aging is associated with a change in PUFAs only, with older males displaying lower PUFA levels in adipose tissue compared to younger males [28].

It is important to seek to understand these differences in women. Some research has suggested it is possibly due to the effect of estrogen. Giltay *et al* [29] observed that females had higher DHA concentrations in serum than men and those consuming oral contraceptives displayed altered fatty acid profiles, suggesting sex hormones may play a role. This may account for differences between pre- and post-menopausal women.

Further differences between pre- and post-menopausal women were observed by Maynar *et al* [30], who examined total plasma fatty acids between these two groups and saw a number of differences including: i) increases in C18:0 and C18:1 in post-menopausal women, ii) decreases in LA and AA in post-menopausal women, and iii) lower 20:4/20:3 desaturase ratio in postmenopausal women, which may have implications on the oxygenated metabolites that are produced from these fatty acids.

It has become clear that when studying lipid metabolism among humans, separating males and females and pre- and post-menopausal women will provide the most accurate and translational results.

Production of Oxygenated Metabolites

Researchers are still trying to understand the precise functions that PUFAs have in the body. One of the ways that PUFAs mediate their function is through the production of oxygenated metabolites. Interest in the area of omega-3 supplementation has provided us with many studies showing overall benefits of supplementation and consumption of omega-3 fatty acids. A better understanding of the oxygenated metabolites that are derived from these omega-3 and omega-6 PUFAs will be beneficial in increasing our understanding of how these PUFAs function in the body.

AA and its oxygenated metabolites have been extensively researched, however, we are only just beginning to understand oxygenated metabolites that arise from the other long chain PUFAs, including EPA and DHA. Additionally, a large gap in the literature exists regarding the production and function of ALA-derived oxygenated metabolites as well as those produced from the precursor to AA, LA.

1.2 Oxylipins

Oxylipins are oxygenated metabolites of PUFAs that are produced via three main enzymatic pathways. Cyclooxygenase (COX) produces the prostaglandins, prostacyclins, and thromboxanes; lipoxygenase (LOX) produces hydroxy fatty acids, resolvins, protectins, lipoxins, and leukotrienes; and the cytochrome P450 (cP450) pathways result in epoxy and hydroxy fatty acids. Oxylipins can be produced from C_{18} fatty acids (octadecanoids), C_{20} fatty acids (eicosanoids), and C_{22} fatty acids (docosanoids) and can be identified in many parts of the body including serum, urine, and tissues. The best-known oxylipins are those derived from AA, a C_{20} fatty acid. These eicosanoids have been extensively studied for more than 40 years. Despite the

large quantity of research that exists regarding the oxylipins produced from AA, much is still unknown regarding the production and functions of those produced from C_{18} and C_{22} fatty acids.

The PUFA precursors of oxylipin synthesis are not typically found in large quantities in their free form within the body; instead they are stored in membrane phospholipids at the *sn*-2 position [31]. Omega-6 and omega-3 PUFAs compete to be esterified at the *sn*-2 position on membrane phospholipids after being absorbed through the gastrointestinal tract and into the blood [32]. These PUFAs are then released from phospholipids in response to extracellular stimuli via phospholipase A_2, which cleaves fatty acids from the *sn*-2 position on the phospholipid backbone. The amount and type of oxylipin produced depends on several things including the amount and type of fatty acid stored as phospholipid and the cleavage enzymes that are present. Once released from the phospholipid, these fatty acids can be rapidly oxygenated to produce lipid mediators by different oxygenating enzymes, depending on which of them is locally present in the membrane. They can also be non-enzymatically converted to certain oxylipins. It is important to note that only PUFAs can be enzymatically or non-enzymatically converted to oxylipins while monounsaturated fatty acids and saturated fatty acids are not substrates for conversion to oxylipins.

Many of the oxylipins produced enzymatically or non-enzymatically are lipid mediators and they are involved in a variety of cellular signaling pathways downstream of G-coupled protein receptors (GCPR) [33], and PPARs [31]. These signaling pathways can release second messengers (i.e. cyclic adenosine monophosphate (cAMP)) to create a physiological response upon oxylipin binding to its receptor. Typically the resulting lipid mediators are produced locally and they act locally (paracrine agents), thus differentiating them from hormones [33].

Generally, oxylipin production is dependent on available substrate for COX, LOX, and cP450. As mentioned above, omega-6 and omega-3 oxylipins also compete for these enzymes to produce their products; in particular competition occurs between AA and EPA due to their similar structures, as both are 20 carbons in length and contain several double bonds [34]. Competition between EPA and AA has many effects in the body including inhibiting the production of omega-6 oxylipins and increasing the production of omega-3 oxylipins [35]. Below we will discuss the products that arise from each of the enzyme classes and the substrates that each enzyme accepts. Further details are included in Appendix 4.1– which is a review paper focusing on oxylipins from dietary PUFA that was written in collaboration with other lab members.

Cyclooxygenase Products

COX enzymes are membrane bound and mainly responsible for the formation of prostanoids, which includes the prostaglandins, prostacyclins and thromboxanes. COX enzymes are able to produce several metabolites by utilizing different enzyme forms including COX-1 and COX-2, which are also known as prostaglandin endoperoxide H synthase 1 and 2, respectively. COX-2 displays wider substrate specificity than COX-1 and in cultured cells it appears to utilize AA, EPA, ALA, GLA, and LA as substrates [36]. Additionally, under certain conditions ALA derived 13-hydroxy-octadecatrienoic acid (13-HOTrE) can be produced by COX [36] and DHA derived 13-hydroxydocosahexaenoic acid (HDoHE) can be produced by COX-2 [37]. The utilization of ALA, and DHA by COX enzymes in humans is unclear in the literature.

Some COX enzymes are constitutively expressed in the body, whereas others are inducible. COX-1, which appears to be responsible for maintaining homeostasis and biological functions, is usually constitutively expressed [38]. On the other hand, COX-2 can be induced and

is often expressed during periods of inflammation; it can also be the target of many non-steroidal anti-inflammatory drugs (NSAID) [38]. NSAIDs, such as aspirin or acetylsalicylic acid (ASA), act as competitive inhibitors at the active sites of both COX enzymes and lead to acetylation of the active site rendering it unable to produce the prostaglandins and thromboxanes, and thereby decreasing inflammation [39]. This targeting of COX enzymes by NSAIDs can also produce products with interesting and novel properties. For example, COX-2, when acetylated, has the capacity to accept DHA as a substrate and in combination with 5-LOX produces the D series resolvins and neuroprotectins, which play an active role in the resolution of inflammation [40]. Acetylated COX-2 can also accept other substrates at sites other than its active site, and they will be outlined below.

Arachidonic Acid Derived Cyclooxygenase Products

When acted on by either COX isoform, AA is converted into prostaglandin G_2 (PGG_2) and trace amounts of 11- or 15-hydroxy-eicosatetraenoic acid (HETE); PGG_2 is then converted into PGH_2, also by COX [41]. PGH_2 is a substrate for many prostaglandin and thromboxane synthases and can be converted into several products including PGI_2 (also known as prostacyclin), thromboxane A_2 (TXA_2), PGE_2, PGD_2, $PGF_{2\alpha}$ [41] as well as 12-hydroxy-heptadecatrienoic acid (HHTrE) [42] (Figure 1-2). Preference of PGH_2 conversion into its downstream metabolites is not yet well understood [43]. These AA COX products are then utilized in their present form or converted into other products via different enzymatic or non-enzymatic reactions, including several that utilize cP450 or LOX enzymes.

The 2-series prostaglandins produced by COX enzymes are responsible for modulating a number of different processes within the body. PGE_2, for example, has been extensively studied and is implicated in many different biological conditions including inflammatory diseases such

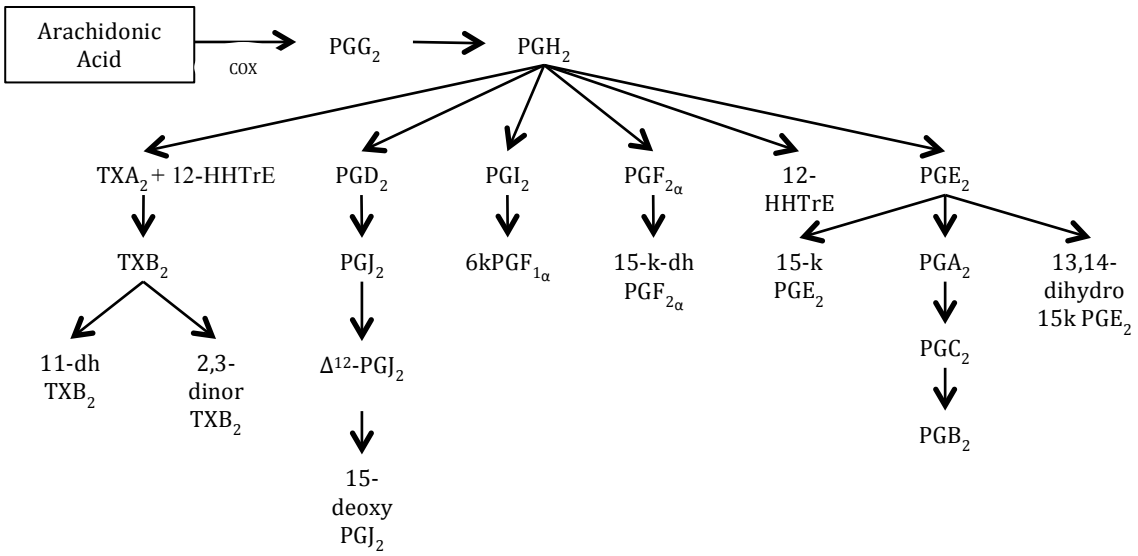


Figure 1-2. Arachidonic acid derived cyclooxygenase oxylipins.
Major metabolites produced from arachidonic acid by cyclooxygenase.

as rheumatoid arthritis and osteoarthritis [43], and it may also be pro-tumorigenic [44]. The metabolite PGI_2, is rapidly converted to the stable but non-biologically active compound 6-keto prostaglandin $F_{1\alpha}$ (6-k $PGF_{1\alpha}$) and is implicated many times in the literature as it plays an important role in the inhibition of platelet aggregation and as a powerful vasodilator [45]. $PGF_{2\alpha}$ is also implicated in a number of different physiological processes including ovulation, renal function, contraction of arteries, brain injury, and pain, as reviewed by Riccotti and FitzGerald [39]. $PGF_{2\alpha}$ also produces a stable metabolite, 15-keto dehydro-prostaglandin $F_{2\alpha}$ (15-k dh-$PGF_{2\alpha}$), which can be found in serum and urine [46]. The role of PGD_2 in the body spans many areas and in some ways is still unclear; it is produced in mast cells and leukocytes and has shown both pro- and anti-inflammatory processes as well as homeostatic functions [47]. For more examples of functions of these prostanoids please see Table 1-1.

PGE_2 and PGD_2 also produce a number of downstream metabolites. PGE_2 can be converted into several different metabolites including 15-keto prostaglandin E_2 (15-k PGE_2), 13,14-dihydro 15-keto prostaglandin E_2 (13,14-dihydro 15-k PGE_2) and PGA_2 [41]. PGA_2 can subsequently be converted into PGC_2, which can then be converted into PGB_2 [41]. PGD_2 is converted into PGJ_2, which is then converted into Δ^{12} PGJ_2, which can then be converted into 15-deoxy $\Delta^{12,14}$-prostaglandin J_2 (15-deoxy PGJ_2) [41]. The conversion of PGD_2 into its metabolites is a very rapid process [47] and the downstream products of PGD_2 appear to be mediators of the anti-inflammatory action that PGD_2 shows [38]. Table 1-1 shows several examples of the functions that these metabolites display.

The TX are a specific type of prostanoid that are produced from AA through COX action that were first identified in 1975 [48]. The thromboxanes include TXA_2, TXB_2, and their degradation products. TXA_2 is the counterbalance signal to PGI_2, and TXB_2 is biologically

inactive [41]. Produced in the platelets, TXA_2 causes irreversible platelet aggregation [48], but has an extremely short half-life of about 30 seconds [39] and is degraded quickly to TXB_2. Further, TXB_2 is degraded to 11-dehydro thromboxane B_2 (11-dh TXB_2) or 2,3-dinor thromboxane B_2 (2,3-dinor TXB_2), which are markers for thromboxane synthesis in plasma and urine respectively [49]. The TX play an important role in blood clotting in the body, but over production may cause an increase in platelet aggregation. For a more comprehensive list of these AA produced COX oxylipins please see Appendix 4.1. A graphical representation of the production of several well-characterized AA COX products can be found in Figure 1-2.

Eicosapentaenoic Acid Derived Cyclooxygenase Products

Both COX-1 and COX-2 have the ability to accept EPA as a substrate for enzymatic activity. COX-1 can oxygenate EPA, but only at 10% the rate of AA, whereas COX-2 has a higher capacity for EPA [50]. EPA COX products include the 3-series PG, prostacyclins, and TX as well as the aspirin-triggered E-series resolvins. The 3-series PG are produced when COX utilizes EPA as a substrate to produce PGH_3, which is converted into TXA_3, $PGF_{3\alpha}$, PGI_3, PGE_3, or PGD_3 and subsequently Δ^{12}-PGJ_3 [16, 51] (Figure 1-3). These products have many beneficial effects in the body and are important in the inflammation process and cancer. Wada *et al.*[50], found that EPA COX products, the 3-series prostanoids, have 2-3 times less potency than their AA two series analogues. PGE_3 shows less potency and is less pro-inflammatory and less pro-angiogenic than PGE_2 [16]. Further, the down stream metabolite Δ^{12} PGJ_3 was shown to limit the progression of leukemia in a mouse model [52]. Table 1-2 displays more specific functions of these EPA derived COX metabolites. Additionally, the aspirin-triggered E-series resolvins are produced when EPA is oxygenated by acetylated COX-2 and then further metabolized by LOX enzymes. This pathway and the function of the E-series resolvins will be discussed further in the

LOX section. For more in-depth information on EPA COX metabolites please see Appendix 4.1. A graphical representation of the production of several well-characterized EPA COX products can be found in Figure 1-3.

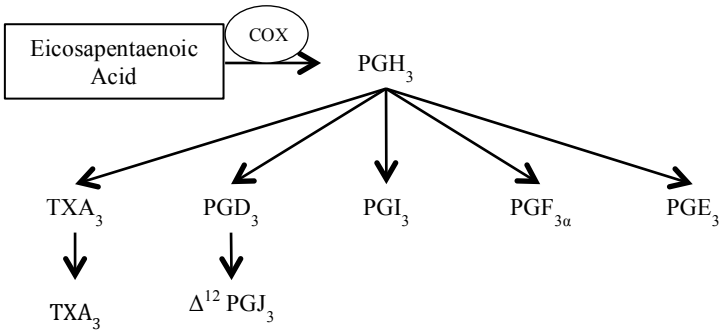


Figure 1-3. Eicosapentaenoic acid derived cyclooxygenase oxylipins.
Major metabolites produced from eicosapentaenoic acid by cyclooxygenase.

Examples of Functions of Major Cyclooxygenase Metabolites

PGE_2	Up-regulates vascular endothelial growth factor expression in MKN28 gastric cancer cells via epidermal growth factor receptor signalling system. Induces cell proliferation in NIH 3T3 fibroblasts	[53] [16]
PGI_2	Relaxes smooth muscle and reduces cell proliferation. Inhibits DNA synthesis in vascular smooth muscle cells Potent vasodilator in humans and animal models	[54] [55] [56]
$PGF_{2\alpha}$	Causes in vitro tachycardia in mouse model Initiates parturition when interacting with ovarian cells in mice	[57] [58]
PGD_2	Inhibits chondrocyte apoptosis Selectively induces eosinophil apoptosis Contributes to homeostasis in the lung	[59] [60] [61]
TXA_2	In vitro, causes irreversible aggregation of human platelets Causes in vitro tachycardia in mouse model	[48] [57]
TXB_2	Biologically inert	[41]
11-dh TXB_2	Major stable metabolite of TX and marker in plasma	[49]
12-HHTrE	Induces activation of extracellular signal-regulated kinases in human prostate cancer cells and lung fibroblasts Accelerates wound healing in mouse model	[62] [63]
PGA_2	Co-stimulates the maturation of human dendritic cells	[64]
6-k PGE_1	Inhibits platelet aggregation in rabbit platelet-rich plasma Stimulates coronary vasodilation in canine model	[65] [66]
15-k PGE_2	Activates PPARγ to enhance adipogenesis of 3T3-L1 cells	[67]
13,14-dihydro-15k-$PGF_{2\alpha}$ (PGFM)	Production increases with labour progression in healthy pregnancy women; related to contraction interval.	[68]
PGJ_2	Selectively induces eosinophil apoptosis Ineffective at targeting leukemia stem cells for apoptosis	[60] [67]
Δ^{12} PGJ_2	Induces apoptosis of leukemia stem cells Induces caspase-dependent apoptosis in eosinophils and neutrophils	[52] [60]
15-deoxy PGJ_2	Inhibits chondrocyte apoptosis Induces caspase-dependent apoptosis in eosinophils and neutrophils Binds to PPARγ to promote cell differentiation	[59] [60] [69]

Table 1-1. Functions of select arachidonic acid derived cyclooxygenase products

PGE_3	Down regulates expression of Akt (cell proliferation) in A549 lung cancer cells Acts as a partial agonist at the PGE_2-EP4 receptor in a colon cancer cell line	[51] [70]
PGI_3	Inhibits platelet aggregation	[71]
$PGF_{3\alpha}$	Less protective than $PGF_{2\alpha}$ on gastric mucosal injury by ethanol	[72]
PGD_3	Inhibits aggregation by increasing cAMP levels in platelets Inhibits migration of neutrophils by antagonizing PGD_2 receptor.	[73] [74]
TXA_3	Interferes with the aggregation of platelets in human platelet-rich-plasma	[75]
Δ^{12}-PGJ_3	Selectively targets leukemia stem cells for apoptosis in the spleen and bone marrow of a mouse model of leukemia	[52]

Table 1-2: Functions of select eicosapentaenoic acid cyclooxygenase products

Lipoxygenase Products

The lipoxygenases accept several different substrates, including AA, LA, ALA, EPA and DHA, to produce a number of different biologically relevant products. Products of the LOX pathways show both pro- and anti-inflammatory actions and are composed of the pro-inflammatory leukotrienes and hydroxy fatty acids and the anti-inflammatory resolvins, protectins, and lipoxins. There are many different LOX enzymes, including 5-LOX, 12-LOX, 15-LOX, and 8-LOX, a murine-derived homologue of the human 15-LOX-2 isozyme [44], that act on different and specific substrates. LOX enzymes are named based on the position of the carbon atom being oxidized by the enzyme from the carboxy-end of AA [76].

Arachidonic Acid Derived 5-Lipoxygenase Products

AA is the most researched LOX substrate and is converted to 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) and subsequently to the unstable leukotriene A_4 (LTA_4) through 5-LOX and 5-LOX activating protein (FLAP) [76, 77]. LTA_4 can then be converted into one of three different analytes. It can produce 5,12-dihydroxy-eicosatetraenoic acid (5,12-DiHETE) [78], otherwise known as LTB_4 through leukotriene A_4 hydrolase, LTC_4 through leukotriene A_4 synthase, or lipoxin A_4 (LXA_4), which will be discussed further below [77]. LTC_4 can then be converted subsequently into LTD_4 and LTE_4; these metabolites are known as the cystienyl leukotrienes and they act as the slow acting components of anaphylaxis [77]. The metabolite 5-HpETE can also be enzymatically converted into 5-HETE, and subsequently into 5,20-DiHETE, which is much less bioactive than 5-HETE [79], 5-oxo-eicosatetraenoic acid (5-oxo-ETE) [35], or non-enzymatically converted into Δ-trans 12-epi LTB_4, 12-epi LTB_4, or Δ^6 trans LTB_4 [41]. Additionally, 5,15-DiHETE can also be produced via a double lipoxygenation

reaction involving 5-LOX and 15-LOX [80]. Please see Figure 1-4 for a graphical representation of some of the above pathways.

The leukotrienes produced from AA are typically regarded as pro-inflammatory and are produced largely by inflammatory cells including polymorphonuclear (PMN) leukocytes, macrophages, and mast cells [33]. The 5-LOX metabolite 5-HETE increases inflammation, angiogenesis, and cancer, as reviewed by Wang *et al.* [16]. LTB_4, produced by 5-LOX, is known to have potent activation of chemotaxis and aggregation and it is a powerful chemoattractant of neutrophils [41, 81]. As mentioned above, LTC_4, LTD_4, and LTE_4 are all slow acting substances of anaphylaxis and have a signaling role in inflammation [77].

One pathway for the production of the non-inflammatory lipoxins starts with the oxygenation of AA by 15-LOX, followed by 5-LOX, and this is seen in a number of different tissues including tissues in the respiratory tract, gastrointestinal tract, and oral cavity [82]. Another pathway produces lipoxins from leukocyte derived LTA_4 through 12-LOX in platelets [83]. Both of these pathways produce the products LXA_4 and LXB_4 (Figure 1-4). LXA_4 and LXB_4 are responsible for inhibiting neutrophil entry into inflamed sites, which leads to resolution of inflammation [83]. LXA_4 targets neutrophils, monocytes, and macrophages and performs specific actions including decreasing pain signals, decreasing angiogenesis and cell proliferation, and increasing phagocytosis and interleukin-10 production [84].

COX-2 acetylated by ASA, or aspirin can also produce oxylipins by utilizing AA and further enzymatic reactions by 5-LOX. If AA is acted upon by acetylated COX-2 the resulting major products are 15R-HETE and the aspirin-triggered lipoxins: 15-epi-LXA_4 and 15-epi-LXB_4, which inhibit neutrophil migration and adhesion to vascular epithelial cells [85]. It is also important to note that these aspirin- triggered lipoxins resist conversion to non-metabolically

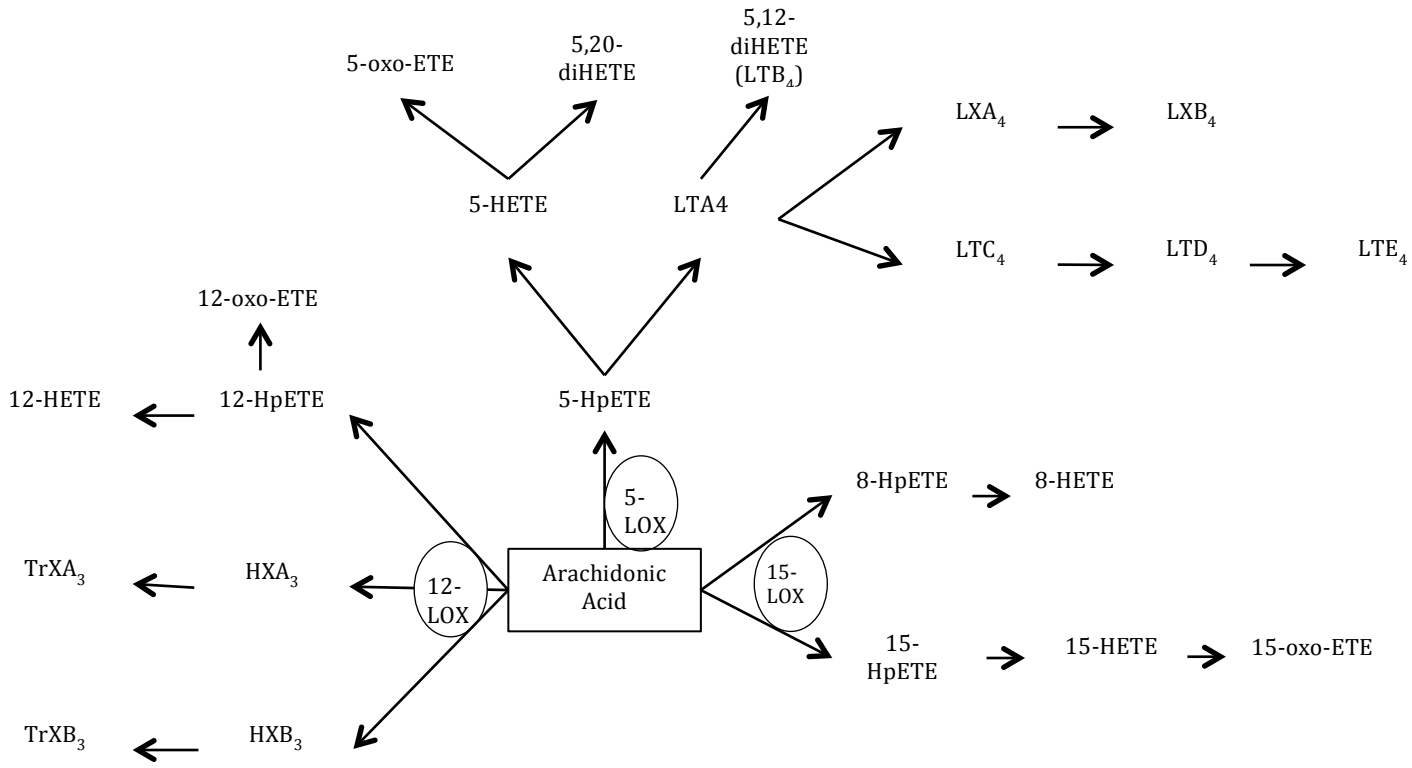


Figure 1-4. Arachidonic acid derived lipoxygenase products.
Major metabolites produced from arachidonic acid by lipoxygenase.

active forms longer than other lipoxins, including LXA_4 and LXB_4 [86].

Arachidonic Acid Derived 15-Lipoxygenase/12-Lipoxygenase Products

AA can also be enzymatically oxygenated by 12-LOX or 15-LOX to produce the lipoxins, 12-HETE and 15-HETE, and the hepoxilins. These LOX enzymes can work alone to convert a substrate into its intended product or they can work in combination other enzymes including other LOX enzymes. AA can be acted upon by 12-LOX to produce hepoxilin A_3 (HXA_3), HXB_3, and 12-HpETE [87]. HXA_3 and HXB_3 can be converted into trioxilin A3 ($TrXA_3$) and $TrXB_3$, respectively [88]. The hepoxilins are biologically active signaling molecules, and HXA_3 in particular causes a number of physiological events including neutrophil migration, and intracellular calcium release [41], however, HXB_3 is biologically inactive [89]. The trioxilins appear to be stable degradation products of the hepoxilins and are found in large quantities in psoriatic scales [90]. The metabolite 12-HpETE is subsequently converted into 12-HETE and further into 12-oxo-ETE [91, 92]. The product 12-HETE and its precursor 12-HpETE display pro-inflammatory actions and when added to 3T3-L1 adipocytes; an increase was seen in several inflammatory markers as well as a decrease in anti-inflammatory adiponectin [93].

The enzyme 15-LOX can also utilize AA to produce several different products. Initially AA can be converted into 8-HpETE or 15-HpETE, both of which are subsequently converted into their corresponding HETE [41]. The metabolite 15-HETE can then be utilized as substrate for 5-LOX to produce LXA_4 or LXB_4 or to produce 15-oxo-ETE, as mentioned above [82]. It is also suggested that 9-HETE is produced by lipoxygenase activity and this has been shown in brain tissue [77]. For more examples of functions of these AA LOX produced oxylipins and their functions please see Table 1-3 and Appendix 4.1. A graphical representation of the production of several well-characterized AA LOX products can be found in Figure 1-4.

Linoleic Acid Derived Lipoxygenase Products

It is much less clear on how LA derived oxylipins are produced as we have only just recently begun to identify them in tissues. Although much is still unknown regarding these octadecanoids, it has been shown that oxylipins derived from LA form from 1/3-2/3 of total oxylipins detected in the human plasma and serum [94-96]. These LA products have been implicated in several pathological conditions including nonalcoholic steato-hepatitis [97] and Alzheimer's dementia [98] as well as a component of oxidized LDL and atherosclerotic plaque [99].

When oxygenated, LA can produce the hydroxy-octadecadienoic acids (HODE); however, the production of these oxylipins is less well known than the conversion of AA to oxylipins, and which enzymatic pathways produce LA oxylipins have not yet been clarified. Some papers indicate that COX enzymes can produce LA oxylipins, particularly 9-HODE [100, 101], however, this research is not conclusive.

More information does exist regarding the production of LOX derived HODEs, and the current literature does suggest that LOX enzymes are responsible for the production of these metabolites and their pathway is outlined here. The initial step in the conversion of LA is by 15-LOX to produce the intermediate 13-hydroperoxy-octadecadienoic acid (13-HpODE), which subsequently produces 13-HODE through a glutathione-dependent mechanism [102]. This 13-HODE can then be converted enzymatically to 13-oxo-octadecadienoic acid (13-oxo-ODE) [103]. HODEs can also be produced non-enzymatically and this will be discussed later.

The HODEs have very important roles within the body and are likely responsible for pro-inflammatory physiological responses, but they may also play a role in the inhibition of cancer cells by inhibiting proliferation and inducing apoptosis, as reviewed by Zuo and Shureiqi [104].

Additionally, the metabolite 13-oxo-ODE has been shown to have anti-inflammatory effects in a line of colon cancer cells [105]. For additional functions of LA LOX derived oxylipins please see Table 1-4 and Appendix 4.1.

Alpha Linolenic Acid Derived Lipoxygenase Products

ALA can be metabolized by 5-LOX and 15-LOX enzymes to produce a number of different products including 9-HOTrE and 13-HOTrE) their respective hydroperoxy-octadecatrienoic acid [106, 107]. Liu, *et al.* [106], also reported that ALA can undergo a double lipoxygenation through 9-HOTrE by human recombinant 15-LOX that results in four isomers of 9,16-dihydroxyoctadecatrienoic acid (9,16-diHOTrE). There are very few studies that provide information regarding the production of oxygenated metabolites derived from ALA and LOX. However, there has been one study that has shown the ALA derived oxylipin 13-HOTrE has anti-inflammatory properties [108]. Please see Table 1-5 and Appendix 4.1 for further information on the functions of LOX derived ALA oxylipins.

Additionally, COX-2 has the ability to utilize ALA as a substrate for oxygenation reactions; however, the evidence for this is limited. Laneuville *et al*. [36] observed that utilizing ALA as a COX-2 substrate resulted in 12-HOTrE, presumably through 12-HpOTrE. However, more research is needed to investigate the role of COX in the production of ALA oxylipins as well as the function of these oxylipins.

Eicosapentaenoic Acid Derived Lipoxygenase Products

There are a number of classes of LOX derived EPA metabolites including hydroxyeicosapentaenoic acids (HEPE), leukotrienes, and resolvins, however, some have been better characterized than others. Several LOX derived HEPE metabolites formed from EPA have been characterized, including 5-HEPE, 12-HEPE, and 15-HEPE. The metabolite 5-HEPE is

produced through 5-LOX, presumably through an EPA derived analogue of HpETE, 5-hydroperoxy-eicosapentaenoic acid (5-HpEPE) [109]. The metabolite 12-HEPE is produced in a similar way through 12-LOX and it can also be converted into 5,12-dihydroxy-eicosapentaenoic acid (5,12-diHEPE) by 5-LOX or non-enzymatically converted into 12,20-diHEPE (Figure 1-5) [110]. Finally, 15-HEPE has been shown to be produced through 15-LOX in guinea pig epidermis [111]. Additionally, LTB_5, which is produced from EPA via 5-LOX, is not as potent as the rest of the leukotrienes, but is still thought to attenuate inflammation [76, 112].

The E series resolvins, resolvin E1 (RvE1) and RvE2, can be produced via two different but related enzymatic pathways. The E-series resolvins and the aspirin-triggered E-series resolvins are produced by utilizing LOX enzymes and acetylated COX-2, respectively. EPA can be first oxygenated by acetylated COX-2 or LOX to produce 18R-HpEPE, which is further converted to 5S-hydroperoxy, 18R-hydroxy-eicosapentaenoic acid [40]. This metabolite is then converted into 5,6-epoxy, 18R-hydroxy-eicosapentaenoic acid and further into RvE1 [40]. The metabolite 5S-hydroperoxy, 18R-hydroxy-eicosapentaenoic acid can also be directly converted into RvE2 [113]. A simplistic version of this pathway is shown in Figure 1-5. Evidence suggests that RvE1 and RvE2 may be biosynthesized by PMNs utilizing the 5-LOX pathway [114].

Resolvins are novel metabolites produced from PUFAs by either LOX or aspirin-triggered COX that were first identified during the resolution phase of acute inflammation, and they were so named because they are “resolution interaction products” [40]. These resolvins have the capabilities to increase monocyte recruitment and decrease neutrophil infiltration, which leads to the resolution of acute inflammation [83]. RvE1 has been shown to initiate resolution of inflammation in exudates sooner than in spontaneous resolution and it has also shown beneficial

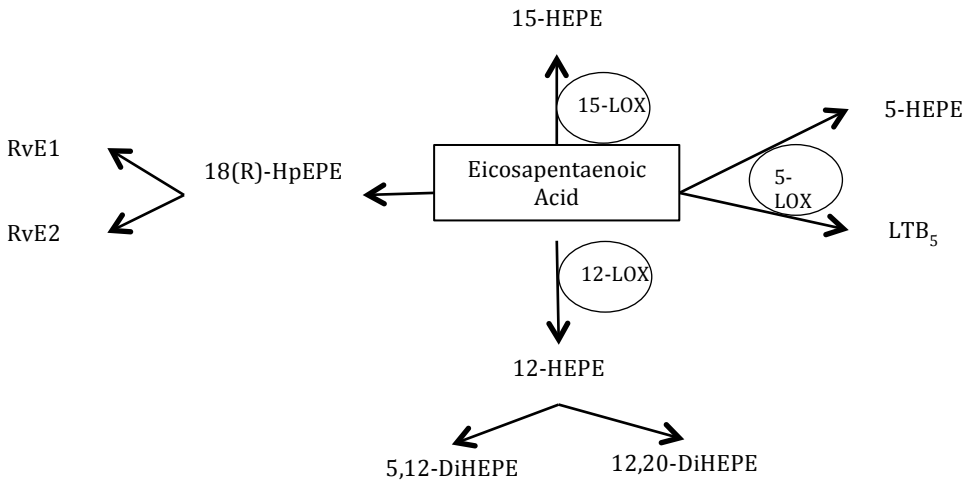


Figure 1-5. Eicosapentaenoic acid derived lipoxygenase products.
Major oxylipins produced from eicosapentaenoic acid by lipoxygenase.

effects in a number of disease conditions in rabbit and mouse models including periodontitis, retinopathy, and colitis, and these are reviewed by Serhan, Chiang, and Van Dyke [83]. The resolvins are novel metabolites and thus more information regarding their role in specific disease condition remains to be elucidated. For further information regarding EPA derived oxylipins produced via LOX enzymes please see Table 1-6 and Appendix 4.1. A graphical representation of the production of several well-characterized EPA LOX products can be found in Figure 1-5.

Docosahexaenoic Acid Derived Lipoxygenase Products

LOX enzymes can produce a number of metabolites utilizing DHA, including hydroxydocosahexaenoic acids (HDoHE), resolvins, protectins, and maresins. A number of other HDoHE metabolites may also derive from LOX, including 4-HDoHE, 7-HDoHE, 11-HDoHE, 14-HDoHE, and 17-HDoHE. Corey *et al.*[115], have shown that 4-HDoHE and 7-HDoHE are produced in small quantities by 5-LOX activity. The metabolites 11-HDoHE and 14-HDoHE are produced via 12-LOX in human platelets [116, 117]. Finally, 15-LOX was shown to produce 17-HDoHE in human platelets, and this metabolite is the precursor for the D series resolvins [118].

DHA can be utilized to produce the D-series resolvins enzymatically via 15-LOX and 5-LOX [119] or via acetylated COX-2 into the aspirin triggered D-series resolvins [37]. The LOX derived D-series resolvins seem to have the same actions as the aspirin-triggered D series resolvins as outlined by Serhan, *et al.*, however, they differ by exhibiting 17-S chirality [37]. Similar to the pathway outlined earlier, the D-series resolvins are produced when DHA is acted upon by LOX to produce 17S-hydroperoxy-docosapentaenoic acid (HpDoHE), which can subsequently be converted to 7S,8S-epoxy-17S-hydroxy-docosahexaenoic acid or 4S,5S-epoxy-17S-hydroxy-docosahexaenoic acid [84]. These products produce RvD1 and RvD2 and RvD3 and RvD4, respectively [84]. Please see Figure 1-6 for a graphical representation of this

pathway. The D series resolvins have a number of different known functions and many are related to decreases and resolution of inflammation [37]. Additionally, RvD1 was found to be beneficial in a number of different disease conditions in mouse models, most notably peritonitis [119], acute kidney injury [120], and retinopathy [121]. Due to the recent discovery of these novel metabolites, much remains unknown regarding the specific functions of RvD1-4 and how they interact in disease conditions.

In addition to the D-series resolvins, DHA can be utilized to produce the D-series protectins/neuroprotectins, docosatrienes, and maresins. To produce the protectins/neuroprotectins and docosatrienes, DHA is oxygenated by 15-LOX to produce 17S-HpDoHE, as in the production of the D series resolvins, however, this product is transformed into 16S, 17S-epoxy-docosahexaenoic acid, and further into a 10,17 dihydroxy product [119], later named 10,17-docosatriene [122]. Please see Figure 1-6 for a graphical representation of this pathway. Following this, the metabolite was named protectin D1 (PD1) or neuroprotectin D1 (NPD1) depending on its site of production, with neuroprotectin being produced in the brain [123]. Additionally a geometric isomer of PD1, called PDX, can also be produced from DHA by LOX action and it has been shown to inhibit platelet aggregation [124]. Maresin 1 (Mar1) and Mar2 are also produced from DHA utilizing 12-LOX in macrophages through the intermediate 13S, 14S-epoxy-maresin [84, 125]. The maresins target monocytes, macrophages, and neutrophils and have specific actions including increasing tissue regeneration and decreasing pain [84].

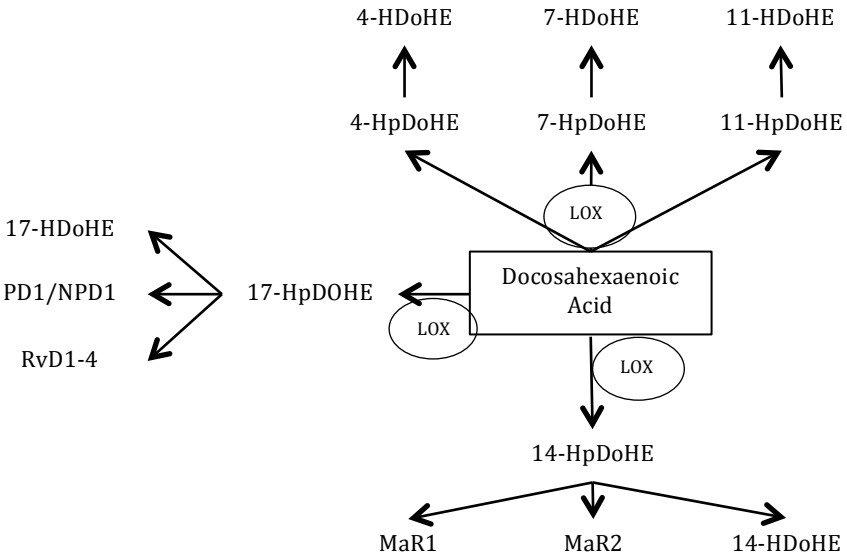


Figure 1-6: Docosahexaenoic acid derived lipoxygenase products.
Major oxylipins produced from docosahexaenoic acid by lipoxygenase.

DHA can also produce resolvins by utilizing acetylated COX-2, and these are known as the aspirin-triggered D-series resolvins and they have 17-R chirality [37]. The pathway of production for the aspirin-triggered D-series resolvins was found in human endothelial cells and begins with acetylated COX-2 producing 17-R-HDoHE [37]. This product has several different fates and can either be utilized to produce the resolvins or PD1/NPD1, which will be further discussed below. To produce the resolvins, 5-LOX utilizes 17R-HDoHE, sometimes referred to as HDHA, to produce either 7R-hydroxyperoxy 17R-hydroxy-docosahexaenoic acid or 4R-hydroperoxy 17R-hydroxy-docosahexaenoic acid [37]. Both of these products go on to produce resolvins through their intermediates 7(8), epoxy 17R-HDoHE and 4(5)-epoxy 17R-HDoHE, respectively, to produce RvD1 and RvD2 and RvD3 and RvD4 [37]. These resolvins appear to have similar functions to EPA LOX derived resolvins when it comes to the resolution of inflammation [126]. For further information regarding DHA derived LOX oxylipins please see Table 1-7 and Appendix 4.1. A graphical representation of the production of several well-characterized DHA LOX products can be found in Figure 1-6.

Examples of Functions of Major Lipoxygenase Metabolites

LTB_4	Releases lysosomal enzymes from PMN Induces PMN aggregation	[127] [128]
LTC_4	Causes release of luteinizing hormone in rat model Mediates inflammation in human skin	[77] [129]
LTD_4	Induces a hyper-response to histamine in bovine airway smooth muscle	[130]
5-HpETE	Inhibits Na+, K(+)-ATPase in rat cerebral cortex	[131]
5-HETE	Induces production of interleukin-8 following treatment of PPARγ Inhibits production of PGI_2 in porcine coronary artery endothelial cells	[105] [132]
5-oxo-ETE	Increases cystolic calcium levels in human neutrophils Stimulates human eosinophil migration.	[35] [133]
LXA_4	Inhibits eosinophil chemotaxis in human peripheral blood leukocytes Suppress tumor growth in mouse hepatocarcinoma cells Potently stimulates monocyte migration and monocyte adhesion in vitro	[134] [124] [135]
LXB_4	Stimulates monocyte migration and monocyte adhesion in vitro Dose-dependently decreased glomerular filtration rate in a rat model	[135] [136]
12-HETE	Increases mitochondrial calcium which leads to an increase in mitochondrial nitric oxide, leading to mitochondrial and oxidative stress Increases monocyte adhesion leading to aortic fatty streak formation	[137] [138]
12-oxo-ETE	Inhibits human platelet 12-LOX and 15-LOX	[139]
HXA_3	Increases calcium in human neutrophils and causes membrane depolarization Recruits PMN to sites of inflammation	[140] [141]
15-HpETE	Exhibits vasodilation or vasoconstriction depending on other vasodilators present	[142]
15-HETE	Contributes to foam cell formation via PPARγ activation in macrophages Inhibits migration of PMN across cytokine-endothelium in vitro	[89, 143] [144]
15-oxo-ETE	Inhibits human platelet 12-LOX	[139]
15-epi LXA_4	Stimulates nitric oxide production and regulates PMN infiltration in humans	[145]
15-epi LXB_4	Stimulates monocyte chemotaxis	[146]

Table 1-3: Functions of select arachidonic acid derived lipoxygenase products

13-HODE	Prevents platelet adherence in vascular epithelium Contributes to foam cell formation via PPARy activation in macrophages Increases monocyte adhesion leading to aortic fatty streak formation	[147] [89, 143] [138]
13-oxo-ODE	Reduces interleukin-8 secretion from HT-29 cells	[105]

Table 1-4: Functions of select linoleic acid lipoxygenase products

13-HOTrE	Suppresses interleukin 1β and induces expression of matrix metalloproteinase-1, -3, and -9 in human chondrocytes in vitro	[108]
9,16-diHOTrE	Decreases prostaglandin synthesis from COX-1 in human platelets Inhibits platelet aggregation triggered by collagen Inhibits COX-2 and may inhibit 5-LOX	[106]

Table 1-5: Functions of select alpha linolenic acid lipoxygenase products

5-HEPE	Agonist for GPCR receptor in pancreatic β-cells and enhances glucose-dependent insulin secretion in human cells	[148]
12-HEPE	Elevated in patients post cardiac surgery; may be implicated in resolution of inflammation	[96, 149]
15-HEPE	Potent inhibitor of 5-LOX in vitro	[111]
LTB_5	Less active than LTB_4 in aggregation and lysosomal enzyme release	[112]
RvE1	Inhibits dendritic cell interleukin-12 production and dendritic cell migration into T cell areas of the spleen. Reduces leukocyte and PMN infiltration in murine exudates	[150] [151]
RvE2	Stops zymogen-induced PMN leukocyte infiltration	[113]

Table 1-6: Functions of select eicosapentaenoic acid lipoxygenase products

4-HDoHE	Directly inhibits endothelial cell proliferation via PPARγ in a mouse model	[152]
Mar1	Mitigates induced liver injury in murine model Decreases PMN infiltration in murine peritonitis	[153] [125]
Mar2	Increases human macrophage phagocytosis of zymosan, but less potently than MaR1	[125]
17-HDoHE	Potent inhibitor of 5-LOX in vitro Reduces genotoxic and oxidative damage in murine hepatocyte cells	[111] [154]
RvD1	Inhibits cytokine interleukin-1β in microglia cells Decreases immunoglobulin E production from human B cells	[37] [155]
PD1	Promotes phagocyte removal during acute inflammation Protects against acute injury in a mouse model	[156] [120]
NPD1	Protects retinal pigment epithelial cells from apoptosis Counteracts leukocyte-mediated injury	[157] [122]

Table 1-7: Functions of select docosahexaenoic acid lipoxygenase products

Cytochrome P450 Products

The cytochrome P450 enzyme group is the third and final group of enzymes that participate in the oxygenation of PUFAs to produce oxylipins in the body. The cP450 enzymes produce oxylipins via two main enzyme classes, ω-hydroxylase and epoxygenase. A number of different cP450 isoforms exist and they all function differently as indicated by their utilization of different substrates and production of different biologically active products. Cytochrome P450 isoforms display different regio-specificity and they are briefly reviewed in Konkel and Schunck [19]. It is interesting to note that several cP450 isoforms accept omega-3 PUFAs as a preferred substrate over omega-6 PUFAs, which may be beneficial to those who have adequate amounts in their diet [81]. The epoxy- products produced through the cP450 epoxygenase enzyme can be altered further by soluble epoxide hydrolase (sEH); this process produces additional biologically relevant products as well as inactivates the initial epoxy products.

Arachidonic Acid Derived Cytochrome P450 Products

AA is a substrate for both the ω-hydroxylase and epoxygenase enzymes and can produce several physiologically active metabolites. The HETEs are produced through ω-hydroxylase enzyme action and the epoxyeicosatetraenoic acids (EpETrE), sometimes seen in the literature as EETS, are produced via epoxygenase. The EpETrE metabolites can then be enzymatically altered through sEH, to produce the dihydroxyeicosatrienoic acids (DiHETrE), also termed DHET. The HETEs are produced through the ω-hydroxylase pathway and they include 16-, 17-, 18-, 19-, and 20-HETE [41] (Figure 1-7). These oxylipins have different and specific functions and several of them have been analyzed individually, see Table 1-8 for examples. Of particular interest is 20-HETE because it is a main metabolite of the ω-hydroxylase enzyme and can display potent pro- and anti-hypertensive action depending on the site of its production [19].

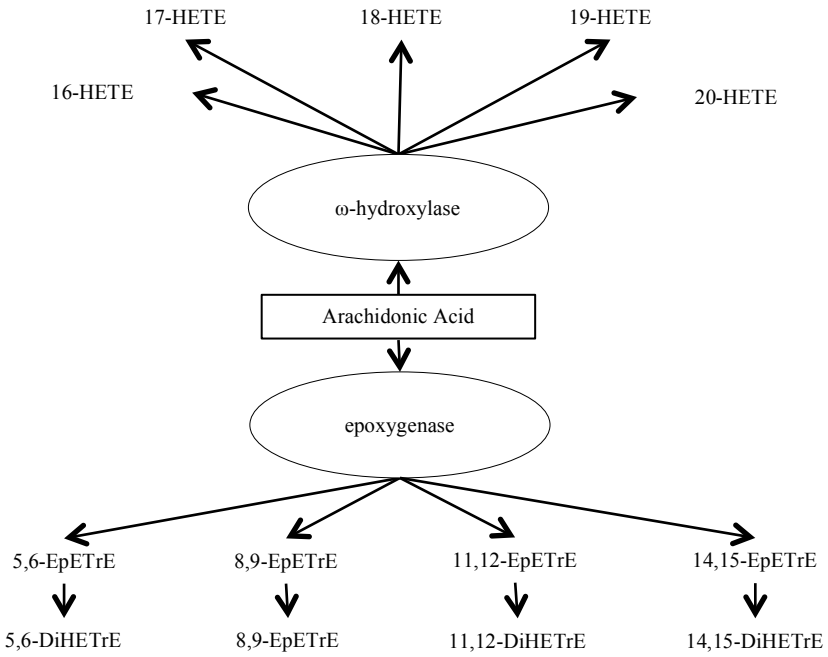


Figure 1-7. Arachidonic acid derived cytochrome P450 oxylipins.
Major oxylipins produced from arachidonic acid by cytochrome P450.

Some of the other HETEs, in particular 19-HETE and 18-HETE, have been shown to inhibit the effects of 20-HETE and induce vasodilation [41]. The ω-hydroxylase enzyme also catalyzes a number of different reactions that utilize prostaglandins and leukotrienes. This process converts LTB_4, PGD_2, PGE_2, and $PGF_{2\alpha}$ into less potent metabolites, which results in 20-OH-LTB_4, 20-OH-PGD_2, 20-OH-PGE_2, and 20-OH-$PGF_{2\alpha}$ [41].

AA can also be utilized by epoxygenase to produce the EpETrE metabolites, including 5,6-EpETrE, 8,9-EpETrE, 11,12-EpETrE, and 14,15-EpETrE [158]. This process occurs in the vascular endothelial cells in response to hormonal stimulation eventually leading to the relaxation of these tissues [19]. The EpETrEs function to lower blood pressure by activating specific channels that lead to the hyperpolarization of vascular smooth muscle and vasorelaxation [158]. The EpETrEs can be subsequently converted into the DiHETrE metabolites by sEH to produce 5,6- DiHETrE, 8,9- DiHETrE, 11,12- DiHETrE, and 14,15- DiHETrE, which are far less potent than their parent EpETrE metabolites [19]. Soluble epoxide hydrolase plays a very important role in converting the EpETrE metabolites to less potent metabolites and researchers are exploring ways to inhibit sEH to prolong the positive effects that the EpETrEs produce [158]. This concept has been explored recently as a means to reduce blood pressure in patients with hypertension by the consumption of muffins containing milled flaxseed as flax contains a natural inhibitor of sEH [159]. Finally, Bannenberg and Serhan also report that AA can be converted to 15R-HpETE through cP450, followed by conversion to 15R-hydroxy-5(6) ETE [151]. This product is then converted through epoxide hydrolase to 15-epi LXA_4 [151]. For further information regarding the AA cP450 products please see Table 1-8 and Appendix 4.1. A graphical representation of the production of several well-characterized AA cP450 products can be found in Figure 1-7.

Linoleic Acid Derived Cytochrome P450 Products

The precursor to AA, LA, can be a substrate for the cP450 family of enzymes. The biologically active HODEs are produced through ω-hydroxylase and the epoxyoctadecamonoenoic acids (EpOME) and dihydroxyoctadecenoic acid (DiHOME) are produced through epoxygenase [160-162]. The resulting metabolites produced from LA through the cP450 family of enzymes are relatively unknown and much work needs to be done in the area to determine the significance of these metabolites.

The ω-hydroxylase enzyme produces 17-HODE and 18-HODE from LA and these enzymes display biological activity but much is still unknown regarding some of their specific functions [160, 161]. More information is known regarding the epoxygenase metabolites, the EpOMEs. The metabolites 9,10-EpOME and 12,13-EpOME are produced from epoxygenase and then rapidly converted into their respective DiHOMEs through sEH. Activated neutrophils have been identified as the main source of the EpOMEs, and they seem to be present during times of acute inflammation, as reviewed by [19]. Please find further information in Table 1-9 and Appendix 4.1.

Alpha Linolenic Acid Derived Cytochrome P450 Products

ALA can also be utilized as a substrate for the cP450 family of enzymes and the major products are the HOTrEs and the epoxy-octadecadienoic acids (EpODE) and their sEH products the dihydroxy-octadecadienoic acids (DiHODE) [19]. Little is known regarding these metabolites and it remains an area to be explored. Epoxygenase and ω-hydroxylase both utilize ALA as substrates and ω-hydroxylase produces 18-HOTrE and epoxygenase produces 9,10-EpODE, 12,13-EpODE, and 15,16-EpODE, which are further hydrolyzed by sEH to their respective DiHODEs [19, 163]. Please see Appendix 4.1 for more information.

Eicosapentaenoic Acid Derived Cytochrome P450 Products

EPA can also be a substrate for cytochrome P450 enzymes: ω-hydroxylase and epoxygenase to produce the epoxy-eicosatetraenoic acids (EpETE), sometimes presented in the literature as EEQ, and HEPEs. Major products through the ω-hydroxylase enzyme include 18-HEPE, 19-HEPE, and 20-HEPE [81, 164], and major products produced through epoxygenase include 8,9-EpETE, 11,12- EpETE, 14,15- EpETE and 17,18- EpETE (Figure 1-8) [19, 81]. The EpETE metabolites can then be hydrolyzed via sEH to produce their corresponding DiHETEs, some times seen as DHEQ. The major epoxy product 17,18-EpETE is known to be a potent vasodilator and this action may contribute to the hypotensive effect of fish oils [165, 166]. Additionally, the E-series resolvins can be produced from the ω-hydroxylase metabolite 18-HEPE [76]. For further information, please see Table 1-10 and Appendix 4.1. A graphical representation of the production of several well characterized EPA cP450 products can be found in Figure 1-8.

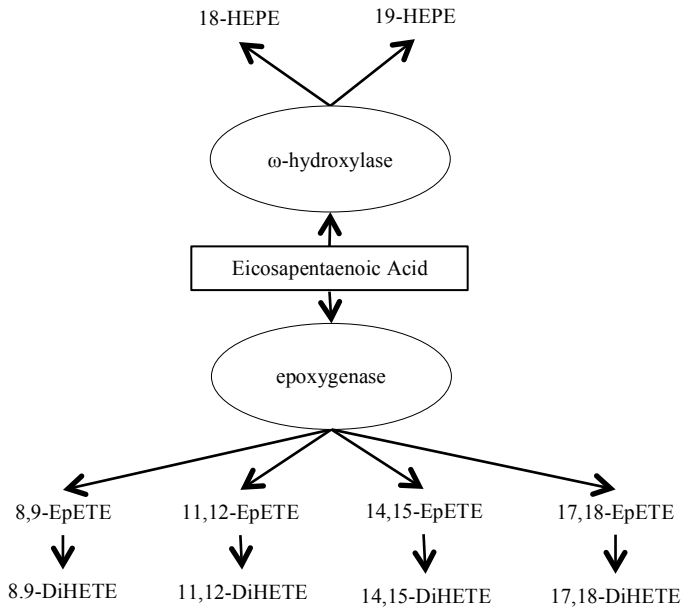


Figure 1-8. Eicosapentaenoic acid derived cytochrome P450 oxylipins.
Major oxylipins produced from eicosapentaenoic acid by cytochrome P450.

Docosahexaenoic Acid Derived Cytochrome P450 Products

The cP450 family of enzymes utilizes DHA as a substrate with both ω-hydroxylase and epoxygenase catalyzing the production of HDoHEs and the epoxydocosapentaenoic acids (EpDPE), respectively. The metabolites 22-HDoHE, 21-HDoHE, and 21,22-HDoHE are produced through the ω-hydroxylase pathway [19, 81, 164] and 7,8-EpDPE, 10,11-EpDPE, 13,14-EpDPE, 16,17-EpDPE, and 19,20-EpDPE are produced utilizing epoxygenase (Figure 1-9) [19, 164]. Soluble epoxide hydrolase can then hydrolyze the EpDPE metabolites into their corresponding dihydroxy-docosapentaenoic acids (DiHDPE), which may play a role in platelet aggregation [19, 167, 168].

It is important to note that EPA and DHA derived cP450 metabolites (EpETrE and EpDPE) may surpass the role of AA derived metabolites as related to vasodilation, as reviewed by Konkel [19]. This may help to identify an important role omega-3 derived oxylipins play in the body. For further information please see Table 1-11 and Appendix 4.1. A graphical representation of the production of several well-characterized DHA cP450 products can be found in Figure 1-9.

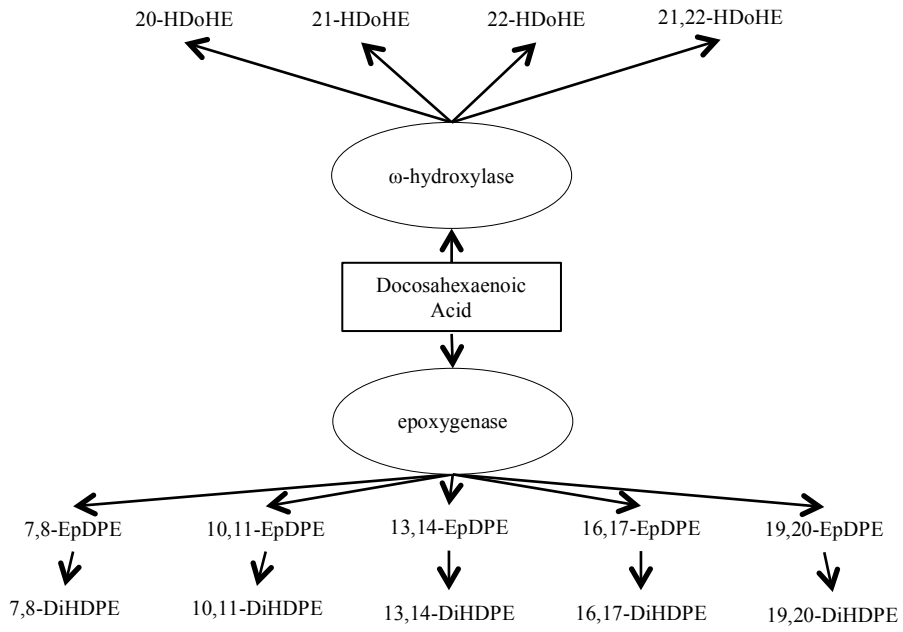


Figure 1-9. Docosahexaenoic acid derived cytochrome P450 oxylipins.
Major oxylipins produced from docosapentaenoic acid by cytochrome P450

Examples of Functions of Major Cytochrome P450 Products

16R-HETE	Induces vasodilation in rabbit kidney tubules In a rabbit model of stroke, suppresses activation of PMN leukocyte and decreases intracranial pressure.	[169] [170]
17-HETE	Reduces sodium reuptake in rabbit kidney model	[169]
18R-HETE	Induces vasodilation in rabbit kidney tubules	[169]
19-HETE	Induces vasodilation in rabbit kidney tubules	[169]
20-HETE	Stimulates production of inflammatory cytokines via nuclear factor-κβ activation in endothelial cells Induces contraction in porcine coronary arteries Promotes salt excretion in the kidney	[171] [172] [173]
5,6-EpETrE	Inhibits nuclear factor-κβ activation leading to decreased inflammation	[174]
8,9- EpETrE	Inhibits apoptosis in bovine aortic endothelial cells Attenuates apoptosis post-exposure to hypoxia and reoxygenation	[175] [176]
11,12-EpETrE	Inhibits basolateral potassium channels in rat renal cortical collecting duct Inhibits nuclear factor-κβ activation leading to decreased inflammation	[177] [174]
14,15-EpETrE	Inhibits apoptosis in bovine aortic endothelial cells Attenuates apoptosis post-exposure to hypoxia and reoxygenation	[175] [176]
5,6-DiHETrE	Induces vasodilation in pre-constricted pressurized mouse arteries	[178]
8,9- DiHETrE	Induces vasodilation in pre-constricted pressurized mouse arteries Vasodilates canine coronary microcirculation	[178] [179]
11,12-DiHETrE	Induces vasodilation in pre-constricted pressurized mouse arteries Vasodilates canine coronary microcirculation	[178] [179]
14,15-DiHETrE	Induces nitric oxide production in mouse aortic endothelial cells Vasodilates canine coronary microcirculation	[178] [179]

Table 1-8: Functions of select arachidonic acid cytochrome P450 products

9,10-EpOME	Decreases mitochondrial respiration rates in perfused rat lung Activates neutrophils	[180] [181]
12,13-EpOME	Maintains mitochondrial respiration in rabbit renal proximal tubular cells Induces vasoconstriction in perfused cat carotid arteries	[182] [183]
9,10-DiHOME	Leads to increases susceptibility of organ injury following ischemia-reperfusion in mouse heart Inhibits active sodium transport in rabbit renal proximal tubular cells	[184] [185]
12,13-DiHOME	Inhibits active sodium transport in rabbit renal proximal tubular cells Toxic mediator involved in the development of acute respiratory distress syndrome in a mouse model	[185] [186]

Table 1-9: Functions of select linoleic acid cytochrome P450 products

18-HEPE	Inhibits activation of murine cardiac fibroblasts leading to inflammation	[187]
8,9-EpETE	Dilates porcine arterioles dependent on concentration Dose dependently inhibits platelet aggregation and thromboxane synthesis	[166] [167]
11,12-EpETE	Dilates porcine arterioles dependent on concentration Dose dependently inhibits platelet aggregation and thromboxane synthesis	[166] [167]
14,15-EpETE	Dilates porcine arterioles dependent on concentration Dose dependently inhibits platelet aggregation and thromboxane synthesis	[166] [167]
17,18-EpETE	Relaxes human bronchi arterial and airway smooth muscles Regulates inflammation in human lungs	[188] [189]
8,9-DiHETE	Weakly inhibits platelet aggregation	[167]
11,12-DiHETE	Weakly inhibits platelet aggregation	[167]
14,15-DiHETE	Weakly inhibits platelet aggregation	[167]
17,18-DiHETE	Weakly inhibits platelet aggregation	[167]

Table 1-10: Functions of select eicosapentaenoic acid cytochrome P450 products

7,8-EpDPE	Potently dilates porcine microvessels Dose dependently inhibits platelet aggregation and thromboxane synthesis	[168] [167]
10,11-EpDPE	Potently dilates porcine microvessels Dose dependently inhibits platelet aggregation and thromboxane synthesis	[168] [167]
13,14- EpDPE	Potently dilates porcine microvessels Dose dependently inhibits platelet aggregation and thromboxane synthesis	[168] [167]
16,17- EpDPE	Potently dilates porcine microvessels Dose dependently inhibits platelet aggregation and thromboxane synthesis	[168] [167]
19,20- EpDPE	Decreases thrombocyte aggregation Inhibits angiogenesis in human endothelial cells	[190] [191]

Table 1-11: Functions of select docosahexaenoic acid cytochrome P450 products

Non-Enzymatically Produced Oxylipins

Many oxylipins can also be produced non-enzymatically within the body from several precursor PUFA. Produced from AA are the PGF_2-isoprostanes, which are produced under biological stress conditions, as reviewed by [46], as well as the cyclopentane isoprostanes by a free radical mediated process as reviewed by [192]. LA contributes largely to the non-enzymatic production of oxylipins and can be utilized to form a number of different products. LA can produce the HODEs non-enzymatically [193, 194] as well as the HpODEs via auto-oxidation and photo-oxidation [195]. Through these processes many LA oxylipins are produced and these non-enzymatically produced products may contribute to the large number of LA oxylipins being found in human plasma and serum [94, 95]. Additionally, non-enzymatically produced oxylipins can be formed from DHA during periods of oxidative stress in the brain in both humans and a rat model, they are referred to as neuroprostanes [196].

Summary of Oxylipin Themes

AA eicosanoids have been well documented and characterized over the years, and so have EPA eicosanoids because of the similarity in structure to AA. Much remains unclear regarding the oxylipins produced from other PUFAs including LA, ALA, and DHA. In particular, it would be of interest to determine the routes of production of the many metabolites of LA and ALA because they are derived from plant based essential fatty acids in the diet of humans. It would also be worthwhile to determine the function of the LA, ALA, and DHA derived oxylipins to better understand the potential pro- and anti-inflammatory roles they may exhibit.

It is suspected that increased AA can contribute to the increased production of pro-inflammatory oxygenated metabolites in the body and LA, in high intakes, may contribute to

chronic inflammation in the body as well via one or more metabolic outcomes: i) it may increase the synthesis of pro-inflammatory oxygenated metabolites from AA; ii) it may inhibit the synthesis of anti-inflammatory oxygenated metabolites from omega-3 PUFA [197]; iii) it may provide a direct substrate for oxygenating enzymes as some LA oxylipins have effects on inflammation as reviewed above. Due to COX, LOX, and cP450 having the ability to accept several different PUFAs as substrates, competition for their active sites occurs [81]. It has been shown that EPA inhibits the oxygenation of AA [50], which may lead to a decrease in the production of inflammatory products. Furthermore, it appears that while AA oxygenated metabolites produced via the LOX pathway are often pro-inflammatory, such as in the case of LTB_4, the EPA metabolites produced via the same pathway, namely LTB_5, are less potent [112]. The same trend is followed when looking at the products from COX enzymes. EPA produces PGE_3 through COX action, which is less potent than its AA analogue PGE_2 [16]. EPA and DHA produce the resolvins and protectins and AA produces the lipoxins via the LOX pathways and these metabolites are potent anti-inflammatory and pro-resolution molecules [40].

Additionally, more research into the area of ALA and DHA supplementation and oxylipin production is required to better understand omega-3 fatty acid conversion as well as understand specifically if there are differences in the oxylipin profiles between sexes and over different time periods of supplementation. While it is suspected that conversion of ALA to DHA is low [22], recent studies have shown that consumption of flax oil, high in ALA, leads to an increase in DHA oxylipins, which suggests DHA may be produced and utilized immediately after production [198] or ALA may affect the activity of oxygenating enzymes.

Understanding the production and functions of oxylipins is important for a number of different reasons. Many studies have shown that the oxylipin profiles of both animals and

humans can be altered if given a diet high in specific PUFAs [3-5, 199]. Once these new dietary fatty acids are incorporated into the phospholipid membrane they can be esterified and utilized to produce oxylipins through COX, LOX, cP450, and through non-enzymatic processes. With this in mind it is of interest to examine at what rate oxylipin production occurs among healthy individuals who are being supplemented different PUFAs to help expand our knowledge of the oxylipin levels present in healthy individuals with the aim of identifying biomarkers for various disease conditions. Further, this knowledge will help to determine the recommended dietary intake of omega-3 fatty acids.

Oxylipin Profiles of Humans

It is important to have an understanding of the human oxylipin profile because oxylipins play such a large role in homeostasis as well as inflammatory processes. There have been two notable works published that look at the human plasma lipidome and serum metabolome [94, 95], however, the oxylipin data presented in both of these is from plasma. Serum and plasma oxylipins may differ because clotting, which is required to obtain serum, is triggered by the release of TXA_2, which is degraded to TXB_2 [94]. Further, concentrations of anticlotting PGI_2 and it's down stream metabolites may differ depending on blood type analyzed, however, this comparison has not been done. It is also important to note that Psychogios *et al.*[94] examined both males and females to determine oxylipin profiles but participants were among a very wide age range (35-65), thereby likely encompassing women who have gone through menopause, which may affect their oxylipin profiles. Further, these samples were not collected at a specific point during the female menstrual cycle, which may also cause variability. There has not been a direct comparison of serum and plasma oxylipins in a healthy young human population.

Further, little additional research exists that examines the oxylipin profiles of healthy humans in depth, and even fewer studies have examined the impact of dietary supplementation with specific PUFAs. In addition to the plasma and serum lipidome studies, Schuchardt *et al.* [96] compared the oxylipin profiles of 20 normolipidemic and 20 hyperlipidemic men but found only minor differences between them. However, this study was carried out only on men thereby producing results that may not be applicable for younger, pre-menopausal women.

Due to recent improvements in HPLC-MS/MS technology we are now able to identify more oxylipins than ever before in human tissue, serum, plasma, and urine and this can help researchers identify and quantify novel metabolites especially ones deriving from LA, ALA, and DHA. In a recent supplementation study, Lundstrom *et al.* [3] were able to screen for 87 oxylipins representing those from COX, LOX, and cP450 pathways. Additionally, in a recent study by Caligiuri *et al.* [200] in our lab, we were able to scan for 81 oxylipins and quantify 38 of them. Most notable is the recent work published by Wang, *et al.*[201] that reported the detection of 184 unique oxylipins.

1.3 Dietary Omega-3 Oil Supplementation

As mentioned above, supplementation with omega-3 oils has become increasingly popular among individuals looking to improve their health status. Supplementing with omega-3 oils results in increased omega-3 fatty acids in plasma and also increases the levels of omega-3 oxylipins in both serum [3] and plasma [5]. This may be because increased omega-3 in the body will substitute for omega-6 in the membrane phospholipids, leading to more omega-3 being incorporated and available for the production of oxylipins. This process is dependent on the source of the omega-3 fatty acids, namely if they are plant based (ALA), or marine based (EPA or DHA) because of the differences in their structure and the differences in the way they are

metabolized in the body. These oxylipins produced from omega-3 fatty acids may be one mechanism by which omega-3 fatty acids exert their beneficial effects in the body. Further, identifying oxylipins that may be present in omega-3 oil will help us to better understand their function as the oxylipin profiles of dietary oils has not been reported in the literature.

Supplementation of Marine Based Omega-3 Fatty Acids

There have been a number of studies published recently that have examined the effects of marine based omega-3 fatty acid supplementation on the oxylipin and fatty acid profiles of humans; however, many of them supplement varying amounts and ratios of EPA and DHA. Many existing studies examine fish oil that contains approximately equal amounts of EPA and DHA [202-204] or fish oil that is composed of EPA to a larger extent [3-5, 205-207], because these are the types of oils that are typically available to consumers. It would be of particular interest to examine supplementation of fish oil that provided a larger ratio of DHA compared to EPA to investigate how oils with this ratio, which is now available on the market, might influence oxylipin profiles. This would provide further understanding of how oxylipin profiles can be impacted by supplementation, and in particular higher doses of DHA.

Supplement dose is also an important consideration when examining changes on oxylipin and fatty acid profiles. Previous research has looked at supplementation with marine omega-3 oils similar to that of the average intake present in a serving of EPA and DHA containing food products, defined by Browning *et al.* [1] as approximately 3.27g/day of EPA + DHA [5, 202-204, 206, 207]. Other research has examined supplementation in larger doses with the aim of identifying benefits of intake beyond the average daily requirement [3, 4, 205].

While there are inadequate data to accurately set a tolerable upper limit for marine omega-3 fatty acids, the European Food Safety Authority, in their scientific opinion paper, concluded that

up to 5 g/day of total EPA and DHA does not present safety concerns in adults [208]. Very few studies examine the oxylipin profile in healthy individuals who are supplemented at levels higher than average daily intake, and virtually none have examined oils with higher ratios of DHA. To further understand the impact of supplementation on both the fatty acid profile as well as the oxylipin profile in healthy individuals, it would be of interest to better understand all ratios of marine omega-3 oils as well as all doses that are deemed safe.

It's important to take supplement dose and composition into consideration when examining supplementation studies, as altering these may result in different changes to the oxylipin profile. A high ratio EPA to DHA oil at intakes higher than average consumption showed increases in several EPA and DHA cP450 derived oxylipins [3, 4], specifically increases in EPA derived 12-HEPE were seen after 3 weeks of treatment [3] and varied results were seen for this analyte after 6 weeks [4]. Further, a high ratio of EPA to DHA oil at intakes similar to average dietary consumption elevated EPA oxylipins after a single dose but caused no changes in DHA derived oxylipins [5]. Treatments with oil containing approximately equal amounts of EPA and DHA at average intake showed increases in EPA and DHA derived oxylipins after 4 weeks [203], while increases in several EPA oxylipins were seen and no changes to DHA oxylipins in a 12 week supplementation [204]. Studies examining high ratio DHA to EPA fish oils have not been seen in the literature and would be an asset to help us further understand how supplementation of DHA impacts the oxylipin profiles.

Supplementation of Plant Based Omega- 3 Fatty Acids

While there have been several studies examining the effect of supplementation of ALA from flax oil on the fatty acid profiles of individuals there are only a few supplementation studies conducted that examined the effect of ALA supplementation on oxylipin profiles. This is

partially because advances in technology have only recently permitted us to identify oxylipin metabolites derived from ALA in the plasma and serum of individuals.

In the studies that have been done, ALA from flax and flax products has been distributed in a number of different ways including by way of flax oil and ground flax seed. Many of these studies have shown increases in plasma ALA during periods of supplementation with flax products. With regards to oxylipin changes, Yamaguchi *et al.* [198] examined a mouse model of polycystic kidney disease and were able to detect increases in 9-HOTrE as well as several EPA and DHA derived oxylipins in the kidney tissue in a group that was fed flaxseed oil compared to diseased animals fed a soy oil diet. Additionally, in a diet-induced obese rat model oxylipin profile alterations were determined between groups that consumed LA verses groups that consumed ALA [199].

When investigating alterations in humans we see increased AA derived 5-HETE and LA derived 9,10,13-trihydroxy-octadecenoic acid (9,10,13-triHOME), and 9,12,13-triHOME in an older population (45-64) than a younger population (19-28); however, these oxylipins decreased to levels similar to those of the young participants after four weeks of a treatment that contained 6 g/day ALA from milled flaxseed [200]. Three oxylipins in the younger group were reported to exhibit greater than or equal to a 2-fold increase in response to ALA treatment (EPA derived 9-HEPE, DHA derived 10-HDoHE, and LA derived 13-oxoODE) [200]. Finally, Caligiuri *et al* found decreases in sEH AA derived 11,12-, and 14,15-DiHETrE and DHA derived 4-HDoHE, 20-HDoHE, 19,20- DiHDPE in hypertensive patients consuming 30 g/day of milled flax seed for 6 months compared to control [159]. Further, ALA derived 9-HOTrE was elevated in patients consuming flax compared to control [159]. Both of these human clinical trials supplement flax in

the form of milled flaxseed and not as a dietary oil supplement; however, no studies that examine flaxseed oil on oxylipin profile are available in the literature.

Previous studies give us a glimpse at what impact supplementation with ALA may have but we lack an understanding of how a larger dose of ALA from a more pure source impacts the oxylipin profile in a younger, healthy population. To further understand how omega-3 supplementation impacts fatty acid profiles and ultimately oxylipin profiles it would be of interest to better characterize the composition of different supplements, understand the impact of different supplementation doses, and focus on a better defined study population.

Participant Characteristics and Oxylipin Profiles

Individuals continue to consume omega-3 supplements and it is important for us to have an understanding of how these supplements impact the oxylipin profiles of healthy individuals with different characteristics. Participant characteristics are also important to examine when comparing clinical studies that supplement individuals with marine-derived omega-3 fatty acids. Some characteristics that could possibly have implications on fatty acid or oxylipin profiles include, sex, age, and menstrual status.

The effect of sex on oxylipin profiles has not been examined to a great extent, and several of the studies examining the effect of omega-3 supplementation on humans include only males, or include females of varying ages. It would be beneficial to specifically include young females in an omega-3 supplementation study in order to accurately determine differences between sexes. As previously mentioned, sex differences in fatty acid metabolism has been documented and it would be of interest to determine if these differences also relate to differences in the oxylipin profile. It has been observed that women have higher circulating DHA levels [209], higher conversion of ALA to DHA [23] and higher production of EPA [23]. Kitson and colleagues

suggested these differences may be in part due to an estrogen effect [210], a particularly important reason why pre-and post-menopausal women should be separated when examining lipid metabolism.

Estrogen levels fluctuate among pre-menopausal women during their reproductive cycle [211] and this could have a contributing impact on how fatty acids are metabolized in the body. The differences between men and women with regards to fat metabolism may ultimately have an effect on the oxylipin profile and it was found that estrogen appears to up regulate COX enzymes, which leads to an increased production of protective PGI_2 in a rabbit model of ischemia-reperfusion [212]. Understanding this phenomenon in humans is important because it has not been examined.

Several studies in the literature have examined oxylipin profiles of males only [5, 96, 206, 207], however, more have examined both males and females [3, 4, 163, 202-205]. This is important because of the previously mentioned differences in lipid metabolism between sexes. Of the studies that included males and females, only three examined sex differences [202, 204, 205] and the two that examined oxylipin profiles [202, 204] found no obvious differences between sexes. However, this may be because they combined older and younger participants there by not taking menopause and the age of women participants into consideration. Alternatively, they examined post-menopausal women who may have similar lipid metabolism as males of the same age due to a decreased effect of estrogen during menopause.

It is also important to note that Nording, *et al.* [4] examined the variations in lipidomic profiles among healthy males and females and found some responses to an omega-3 supplement containing EPA and DHA were consistent, but there was a high degree of variability between subjects. This has many implications because individuals who are being supplemented with a

certain dose of omega-3 fatty acids may display different oxylipin profiles, causing inconsistent results. This suggests it may also be important to take sex into account when discussing the oxylipin profiles of humans who are being supplemented with omega-3 fatty acids.

Age of Participants

The age of the selected participants in these omega-3 oil trials is especially important in those that included females as mentioned above. Shearer *et al*. [163] and Keenan *et al.* [203] included participants whose ages ranged from their early twenties to their late fifties. Having this large of an age range could cause major problems in the consistency and applicability of these results. Older women included in these studies may have varying levels of circulating estrogen, which may alter fatty acid metabolism leading to changes in the oxylipin profiles of these participants [201]. In the study conducted by Nording *et al.* [4] younger women (21-33) were examined along side men of varying ages (26-55) and high variability was observed among participants. This may be explained by the high variability in ages or because menstrual cycle was not taken into account when collecting samples from female participants.

Furthermore, Caligiuri *et al.* [200] found that participants aged 45-64 had 13 oxylipins in significantly higher proportions compared to a younger group aged 19-28, including the pro-inflammatory oxylipin 5-HETE. It thus appears essential that participants be separated by age to better understand changes in oxylipin profiles. Examining the fatty acid, but particularly the oxylipin profiles of healthy, young, males and females to marine- and plant-based omega-3 supplementation will help in understanding the impact these supplements have in the body.

Time Course Changes of Oxylipin and Fatty Acid Profiles

Few studies have examined the time course changes on oxylipin profiles in human serum or plasma, however, several studies have looked at oxylipin changes after a period of several

weeks [3, 4, 197, 210]. It is important to understand the time course changes for the oxylipin and fatty acid profiles because this information could ultimately have an effect on the dietary recommendations for plant and marine based omega-3 fatty acids. It also gives us a better understanding of the rate at which oxylipins are being produced and degraded while consuming omega-3 fatty acids and if there are differences between sexes.

Further, Schuchardt *et al.* [5] have examined the effect of a single dose of EPA and DHA on healthy males over the course of 48 hours and examined the plasma lipid profile at several time points during that time period. Their data suggest there is a rapid conversion to hydroxy, epoxy, and dihydroxy fatty acid metabolites, especially for metabolites from EPA, and they found little change in AA, DHA, and other precursor PUFA [5]. A longer supplementation period or a larger supplementation dose may have had different effects on the oxylipin profile, or as the rate of incorporation of these omega-3 fatty acids into phospholipids. It would have been of interest to examine these changes between sexes.

From examining the research, it appears that no study has looked at changes in the oxylipin profile of humans supplemented with different types of omega-3 fatty acids at higher doses over a period of 4 weeks while systematically examining several short and long term time points. Utilizing novel HPLC-MS/MS technology we can now identify more oxylipins. Further, this technology will help us examine the effect of time course on oxylipin production in human serum so we can identify how long it may take to cause significant changes in oxylipin production, if there are differences between sexes, if any correlation exists between time points, and how fast these changes occur.

1.4 References for Chapter 1

1. Browning, L.M., et al., *Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish.* Am J Clin Nutr, 2012. 96(4): p. 748-58.
2. Lagarde, M., et al., *Lipidomics of essential fatty acids and oxygenated metabolites.* Mol Nutr Food Res, 2013. 57(8): p. 1347-58.
3. Lundstrom, S.L., et al., *Lipid mediator serum profiles in asthmatics significantly shift following dietary supplementation with omega-3 fatty acids.* Mol Nutr Food Res, 2013. 57(8): p. 1378-89.
4. Nording, M.L., et al., *Individual variation in lipidomic profiles of healthy subjects in response to omega-3 Fatty acids.* PLoS One, 2013. 8(10): p. e76575.
5. Schuchardt, J.P., et al., *Increase of EPA-derived hydroxy, epoxy and dihydroxy fatty acid levels in human plasma after a single dose of long-chain omega-3 PUFA.* Prostaglandins Other Lipid Mediat, 2014. 109-111: p. 23-31.
6. Harris, W.S., et al., *Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention.* Circulation, 2009. 119(6): p. 902-7.
7. Chowdhury, R., et al., *Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis.* Ann Intern Med, 2014. 160(6): p. 398-406.
8. Beam, J., et al., *Excess Linoleic Acid Increases Collagen I/III Ratio and "Stiffens" the Heart Muscle Following High Fat Diets.* J Biol Chem, 2015. 290(38): p. 23371-84.
9. Ghosh, S., et al., *Diets rich in n-6 PUFA induce intestinal microbial dysbiosis in aged mice.* Br J Nutr, 2013. 110(3): p. 515-23.
10. Ramsden, C.E., et al., *Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis.* BMJ, 2013. 346: p. e8707.
11. Kris-Etherton, P.M., et al., *Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.* Arterioscler Thromb Vasc Biol, 2003. 23(2): p. e20-30.
12. Marik, P.E. and J. Varon, *Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review.* Clin Cardiol, 2009. 32(7): p. 365-72.
13. Mozaffarian, D. and J.H. Wu, *Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events.* J Am Coll Cardiol, 2011. 58(20): p. 2047-67.
14. Fleming, J.A. and P.M. Kris-Etherton, *The evidence for alpha-linolenic acid and cardiovascular disease benefits: Comparisons with eicosapentaenoic acid and docosahexaenoic acid.* Adv Nutr, 2014. 5(6): p. 863S-76S.
15. Institute of Medicine (U.S.). Panel on Macronutrients and Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. 2005, Washington, D.C.: National Academies Press. xxv, 1-972, 1259-1331 p.

16. Wang, W., et al., *omega-3 polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer.* Prostaglandins Other Lipid Mediat, 2014. 113-115: p. 13-20.
17. *Linoleic Acid Metabolism*. 2013, December 25 [cited 2014 June 11]; Available from: http://www.genome.jp/kegg-bin/show_pathway?map00591.
18. Zhang, J.Y., K.S. Kothapalli, and J.T. Brenna, *Desaturase and elongase-limiting endogenous long-chain polyunsaturated fatty acid biosynthesis.* Curr Opin Clin Nutr Metab Care, 2016. 19(2): p. 103-10.
19. Konkel, A. and W.H. Schunck, *Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids.* Biochim Biophys Acta, 2011. 1814(1): p. 210-22.
20. Papanikolaou, Y., et al., *U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003-2008.* Nutr J, 2014. 13: p. 31.
21. Burdge, G.C. and P.C. Calder, *Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults.* Reprod Nutr Dev, 2005. 45(5): p. 581-97.
22. Brenna, J.T., *Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man.* Curr Opin Clin Nutr Metab Care, 2002. 5(2): p. 127-32.
23. Burdge, G.C. and S.A. Wootton, *Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women.* Br J Nutr, 2002. 88(4): p. 411-20.
24. Hartweg, J., et al., *Meta-analysis of the effects of n-3 polyunsaturated fatty acids on lipoproteins and other emerging lipid cardiovascular risk markers in patients with type 2 diabetes.* Diabetologia, 2007. 50(8): p. 1593-602.
25. Davidson, M.H., *Omega-3 fatty acids: new insights into the pharmacology and biology of docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid.* Curr Opin Lipidol, 2013. 24(6): p. 467-74.
26. Kelley, D.S., et al., *DHA supplementation decreases serum C-reactive protein and other markers of inflammation in hypertriglyceridemic men.* J Nutr, 2009. 139(3): p. 495-501.
27. Mori, T.A., et al., *Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men.* Circulation, 2000. 102(11): p. 1264-9.
28. Tavendale, R., et al., *Adipose tissue fatty acids in Scottish men and women: results from the Scottish Heart Health Study.* Atherosclerosis, 1992. 94(2-3): p. 161-9.
29. Giltay, E.J., et al., *Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects.* Am J Clin Nutr, 2004. 80(5): p. 1167-74.
30. Maynar, M., et al., *Menopause-induced changes in lipid fractions and total fatty acids in plasma.* Endocr Res, 2001. 27(3): p. 357-65.
31. Gurr, M., Harwood, J., & Frayn, K. , *Lipid biochemistry: An introduction*. 5th ed. ed. 2002, Bodmin, Cornwall: MPG Books.
32. Arterburn, L.M., E.B. Hall, and H. Oken, *Distribution, interconversion, and dose response of n-3 fatty acids in humans.* Am J Clin Nutr, 2006. 83(6 Suppl): p. 1467S-1476S.
33. Funk, C.D., *Prostaglandins and leukotrienes: advances in eicosanoid biology.* Science, 2001. 294(5548): p. 1871-5.
34. Lands, W.E., *Biochemistry and physiology of n-3 fatty acids.* FASEB J, 1992. 6(8): p. 2530-6.

35. Powell, W.S., S. Gravel, and F. Gravelle, *Formation of a 5-oxo metabolite of 5,8,11,14,17-eicosapentaenoic acid and its effects on human neutrophils and eosinophils.* J Lipid Res, 1995. 36(12): p. 2590-8.
36. Laneuville, O., et al., *Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2. Formation of 12-hydroxy-(9Z, 13E/Z, 15Z)-octadecatrienoic acids from alpha-linolenic acid.* J Biol Chem, 1995. 270(33): p. 19330-6.
37. Serhan, C.N., et al., *Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals.* J Exp Med, 2002. 196(8): p. 1025-37.
38. Gilroy, D.W., et al., *Inducible cyclooxygenase may have anti-inflammatory properties.* Nat Med, 1999. 5(6): p. 698-701.
39. Ricciotti, E. and G.A. FitzGerald, *Prostaglandins and inflammation.* Arterioscler Thromb Vasc Biol, 2011. 31(5): p. 986-1000.
40. Serhan, C.N., *Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotectins.* Curr Opin Clin Nutr Metab Care, 2005. 8(2): p. 115-21.
41. Buczynski, M.W., D.S. Dumlao, and E.A. Dennis, *Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology.* J Lipid Res, 2009. 50(6): p. 1015-38.
42. Shen, R.F. and H.H. Tai, *Immunoaffinity purification and characterization of thromboxane synthase from porcine lung.* J Biol Chem, 1986. 261(25): p. 11592-9.
43. Park, J.Y., M.H. Pillinger, and S.B. Abramson, *Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases.* Clin Immunol, 2006. 119(3): p. 229-40.
44. Schneider, C. and A. Pozzi, *Cyclooxygenases and lipoxygenases in cancer.* Cancer Metastasis Rev, 2011. 30(3-4): p. 277-94.
45. Kelton, J.G. and M.A. Blajchman, *Prostaglandin I2 (prostacyclin).* Can Med Assoc J, 1980. 122(2): p. 175-9.
46. Basu, S., *Novel cyclooxygenase-catalyzed bioactive prostaglandin F2alpha from physiology to new principles in inflammation.* Med Res Rev, 2007. 27(4): p. 435-68.
47. Sandig, H., J.E. Pease, and I. Sabroe, *Contrary prostaglandins: the opposing roles of PGD2 and its metabolites in leukocyte function.* J Leukoc Biol, 2007. 81(2): p. 372-82.
48. Hamberg, M., J. Svensson, and B. Samuelsson, *Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides.* Proc Natl Acad Sci U S A, 1975. 72(8): p. 2994-8.
49. Catella, F., et al., *11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation.* Proc Natl Acad Sci U S A, 1986. 83(16): p. 5861-5.
50. Wada, M., et al., *Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products.* J Biol Chem, 2007. 282(31): p. 22254-66.
51. Yang, P., Y. Jiang, and S.M. Fischer, *Prostaglandin E3 metabolism and cancer.* Cancer Lett, 2014. 348(1-2): p. 1-11.
52. Hegde, S., et al., *Delta12-prostaglandin J3, an omega-3 fatty acid-derived metabolite, selectively ablates leukemia stem cells in mice.* Blood, 2011. 118(26): p. 6909-19.
53. Ding, Y.B., et al., *PGE2 up-regulates vascular endothelial growth factor expression in MKN28 gastric cancer cells via epidermal growth factor receptor signaling system.* Exp Oncol, 2005. 27(2): p. 108-13.

54. Dorris, S.L. and R.S. Peebles, Jr., *PGI2 as a regulator of inflammatory diseases.* Mediators Inflamm, 2012. 2012: p. 926968.
55. Libby, P., S.J. Warner, and G.B. Friedman, *Interleukin 1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids.* J Clin Invest, 1988. 81(2): p. 487-98.
56. Moncada, S. and J.R. Vane, *Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls.* N Engl J Med, 1979. 300(20): p. 1142-7.
57. Takayama, K., et al., *Thromboxane A2 and prostaglandin F2alpha mediate inflammatory tachycardia.* Nat Med, 2005. 11(5): p. 562-6.
58. Sugimoto, Y., et al., *Failure of parturition in mice lacking the prostaglandin F receptor.* Science, 1997. 277(5326): p. 681-3.
59. Relic, B., et al., *15-deoxy-delta12,14-prostaglandin J2 inhibits Bay 11-7085-induced sustained extracellular signal-regulated kinase phosphorylation and apoptosis in human articular chondrocytes and synovial fibroblasts.* J Biol Chem, 2004. 279(21): p. 22399-403.
60. Ward, C., et al., *Prostaglandin D2 and its metabolites induce caspase-dependent granulocyte apoptosis that is mediated via inhibition of I kappa B alpha degradation using a peroxisome proliferator-activated receptor-gamma-independent mechanism.* J Immunol, 2002. 168(12): p. 6232-43.
61. Hammad, H., et al., *Prostaglandin D2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1.* J Immunol, 2003. 171(8): p. 3936-40.
62. Li, X., J. Wei, and H.H. Tai, *Activation of extracellular signal-regulated kinase by 12-hydroxyheptadecatrienoic acid in prostate cancer PC3 cells.* Arch Biochem Biophys, 2007. 467(1): p. 20-30.
63. Liu, M., et al., *12-Hydroxyheptadecatrienoic acid promotes epidermal wound healing by accelerating keratinocyte migration via the BLT2 receptor.* J Exp Med, 2014. 211(6): p. 1063-78.
64. Thurnher, M., et al., *The cyclopentenone prostaglandin PGA2 costimulates the maturation of human dendritic cells.* Exp Hematol, 2005. 33(2): p. 144-50.
65. Pieroni, J.P., et al., *Identification of 6-keto-prostaglandin E1 obtained from isolated perfused kidney of the rabbit.* J Pharmacol Exp Ther, 1988. 247(1): p. 63-8.
66. Panzenbeck, M.J., T.H. Hintze, and G. Kaley, *6-Keto-prostaglandin E1 is a potent coronary vasodilator and stimulates a vagal reflex in dogs.* J Pharmacol Exp Ther, 1988. 244(3): p. 814-9.
67. Chou, W.L., et al., *Identification of a novel prostaglandin reductase reveals the involvement of prostaglandin E2 catabolism in regulation of peroxisome proliferator-activated receptor gamma activation.* J Biol Chem, 2007. 282(25): p. 18162-72.
68. Nakayama, K., et al., *Changes in 13, 14-dihydro-15-keto-prostaglandin F2alpha levels in saliva during pregnancy, labor and the postpartum period.* J Obstet Gynaecol Res, 2010. 36(1): p. 27-33.
69. Kliewer, S.A., et al., *A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation.* Cell, 1995. 83(5): p. 813-9.

70. Hawcroft, G., et al., *Effect of eicosapentaenoic acid on E-type prostaglandin synthesis and EP4 receptor signaling in human colorectal cancer cells.* Neoplasia, 2010. 12(8): p. 618-27.
71. Kobzar, G., et al., *Comparison of anti-aggregatory effects of PGI2, PGI3 and iloprost on human and rabbit platelets.* Cell Physiol Biochem, 2001. 11(5): p. 279-84.
72. Faust, T.W., et al., *Effect of prostaglandin F3 alpha on gastric mucosal injury by ethanol in rats: comparison with prostaglandin F2 alpha.* Prostaglandins, 1989. 37(4): p. 493-504.
73. Whitaker, M.O., et al., *Triene prostaglandins: prostaglandin D3 and icosapentaenoic acid as potential antithrombotic substances.* Proc Natl Acad Sci U S A, 1979. 76(11): p. 5919-23.
74. Tull, S.P., et al., *Omega-3 Fatty acids and inflammation: novel interactions reveal a new step in neutrophil recruitment.* PLoS Biol, 2009. 7(8): p. e1000177.
75. Needleman, P., et al., *Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties.* Proc Natl Acad Sci U S A, 1979. 76(2): p. 944-8.
76. Hersberger, M., *Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins.* Clin Chem Lab Med, 2010. 48(8): p. 1063-73.
77. Samuelsson, B., et al., *Leukotrienes and lipoxins: structures, biosynthesis, and biological effects.* Science, 1987. 237(4819): p. 1171-6.
78. Bokoch, G.M. and P.W. Reed, *Effect of various lipoxygenase metabolites of arachidonic acid on degranulation of polymorphonuclear leukocytes.* J Biol Chem, 1981. 256(11): p. 5317-20.
79. O'Flaherty, J.T., et al., *Metabolism of 5-hydroxyicosatetraenoate by human neutrophils: production of a novel omega-oxidized derivative.* J Immunol, 1986. 137(10): p. 3277-83.
80. Maas, R.L., et al., *Formation of a novel dihydroxy acid from arachidonic acid by lipoxygenase-catalyzed double oxygenation in rat mononuclear cells and human leukocytes.* J Biol Chem, 1982. 257(12): p. 7056-67.
81. Arnold, C., et al., *Cytochrome P450-dependent metabolism of omega-6 and omega-3 long-chain polyunsaturated fatty acids.* Pharmacol Rep, 2010. 62(3): p. 536-47.
82. Serhan, C.N., *Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution.* Prostaglandins Leukot Essent Fatty Acids, 2005. 73(3-4): p. 141-62.
83. Serhan, C.N., N. Chiang, and T.E. Van Dyke, *Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators.* Nat Rev Immunol, 2008. 8(5): p. 349-61.
84. Buckley, C.D., D.W. Gilroy, and C.N. Serhan, *Proresolving lipid mediators and mechanisms in the resolution of acute inflammation.* Immunity, 2014. 40(3): p. 315-27.
85. Claria, J. and C.N. Serhan, *Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions.* Proc Natl Acad Sci U S A, 1995. 92(21): p. 9475-9.
86. Serhan, C.N., et al., *Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils.* Biochemistry, 1995. 34(44): p. 14609-15.
87. Pace-Asciak, C.R., E. Granstrom, and B. Samuelsson, *Arachidonic acid epoxides. Isolation and structure of two hydroxy epoxide intermediates in the formation of 8,11,12- and 10,11,12-trihydroxyeicosatrienoic acids.* J Biol Chem, 1983. 258(11): p. 6835-40.

88. Pace-Asciak, C.R., *Formation of hepoxilin A4, B4 and the corresponding trioxilins from 12(S)-hydroperoxy-5,8,10,14,17-icosapentaenoic acid.* Prostaglandins Leukot Med, 1986. 22(1): p. 1-9.
89. Dobrian, A.D., et al., *Functional and pathological roles of the 12- and 15-lipoxygenases.* Prog Lipid Res, 2011. 50(1): p. 115-31.
90. Anton, R., et al., *Occurrence of hepoxilins and trioxilins in psoriatic lesions.* J Invest Dermatol, 1998. 110(4): p. 303-10.
91. Fruteau de Laclos, B., et al., *Conversion of arachidonic acid into 12-oxo derivatives in human platelets. A pathway possibly involving the heme-catalysed transformation of 12-hydroperoxy-eicosatetraenoic acid.* Prostaglandins, 1987. 33(3): p. 315-37.
92. Sutherland, M., et al., *Evidence for the presence of phospholipid hydroperoxide glutathione peroxidase in human platelets: implications for its involvement in the regulatory network of the 12-lipoxygenase pathway of arachidonic acid metabolism.* Biochem J, 2001. 353(Pt 1): p. 91-100.
93. Chakrabarti, S.K., et al., *12/15-lipoxygenase products induce inflammation and impair insulin signaling in 3T3-L1 adipocytes.* Obesity (Silver Spring), 2009. 17(9): p. 1657-63.
94. Psychogios, N., et al., *The human serum metabolome.* PLoS One, 2011. 6(2): p. e16957.
95. Quehenberger, O. and E.A. Dennis, *The human plasma lipidome.* N Engl J Med, 2011. 365(19): p. 1812-23.
96. Schuchardt, J.P., et al., *Comparison of free serum oxylipin concentrations in hyper- vs. normolipidemic men.* Prostaglandins Leukot Essent Fatty Acids, 2013. 89(1): p. 19-29.
97. Feldstein, A.E., et al., *Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis.* J Lipid Res, 2010. 51(10): p. 3046-54.
98. Yoshida, Y., et al., *Hydroxyoctadecadienoic acid and oxidatively modified peroxiredoxins in the blood of Alzheimer's disease patients and their potential as biomarkers.* Neurobiol Aging, 2009. 30(2): p. 174-85.
99. Shibata, N., et al., *Immunohistochemical detection of 13(R)-hydroxyoctadecadienoic acid in atherosclerotic plaques of human carotid arteries using a novel specific antibody.* Acta Histochem Cytochem, 2009. 42(6): p. 197-203.
100. Engels, F., H. Willems, and F.P. Nijkamp, *Cyclooxygenase-catalyzed formation of 9-hydroxylinoleic acid by guinea pig alveolar macrophages under non-stimulated conditions.* FEBS Lett, 1986. 209(2): p. 249-53.
101. Kaduce, T.L., et al., *Formation of 9-hydroxyoctadecadienoic acid from linoleic acid in endothelial cells.* J Biol Chem, 1989. 264(12): p. 6823-30.
102. Reinaud, O., et al., *Oxidative metabolism of linoleic acid by human leukocytes.* Biochem Biophys Res Commun, 1989. 161(2): p. 883-91.
103. Bull, A.W., S.M. Earles, and J.C. Bronstein, *Metabolism of oxidized linoleic acid: distribution of activity for the enzymatic oxidation of 13-hydroxyoctadecadienoic acid to 13-oxooctadecadienoic acid in rat tissues.* Prostaglandins, 1991. 41(1): p. 43-50.
104. Zuo, X. and I. Shureiqi, *Eicosanoid profiling in colon cancer: emergence of a pattern.* Prostaglandins Other Lipid Mediat, 2013. 104-105: p. 139-43.
105. Altmann, R., et al., *13-Oxo-ODE is an endogenous ligand for PPARgamma in human colonic epithelial cells.* Biochem Pharmacol, 2007. 74(4): p. 612-22.
106. Liu, M., et al., *Characterization and biological effects of di-hydroxylated compounds deriving from the lipoxygenation of ALA.* J Lipid Res, 2013. 54(8): p. 2083-94.

107. Galliard, T. and D.R. Phillips, *Lipoxygenase from potato tubers. Partial purification and properties of an enzyme that specifically oxygenates the 9-position of linoleic acid.* Biochem J, 1971. 124(2): p. 431-8.
108. Schulze-Tanzil, G., et al., *Effects of the antirheumatic remedy hox alpha--a new stinging nettle leaf extract--on matrix metalloproteinases in human chondrocytes in vitro.* Histol Histopathol, 2002. 17(2): p. 477-85.
109. Kulkarni, P.S. and B.D. Srinivasan, *Eicosapentaenoic acid metabolism in human and rabbit anterior uvea.* Prostaglandins, 1986. 31(6): p. 1159-64.
110. von Schacky, C., et al., *Platelet-neutrophil interactions. 12S,20- and 5S,12S-dihydroxyeicosapentaenoic acids: two novel neutrophil metabolites from platelet-derived 12S-hydroxyeicosapentaenoic acid.* J Lipid Res, 1990. 31(5): p. 801-10.
111. Miller, C., R.Y. Yamaguchi, and V.A. Ziboh, *Guinea pig epidermis generates putative anti-inflammatory metabolites from fish oil polyunsaturated fatty acids.* Lipids, 1989. 24(12): p. 998-1003.
112. Terano, T., J.A. Salmon, and S. Moncada, *Biosynthesis and biological activity of leukotriene B5.* Prostaglandins, 1984. 27(2): p. 217-32.
113. Tjonahen, E., et al., *Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis.* Chem Biol, 2006. 13(11): p. 1193-202.
114. Oh, S.F., et al., *Pro-resolving actions and stereoselective biosynthesis of 18S E-series resolvins in human leukocytes and murine inflammation.* J Clin Invest, 2011. 121(2): p. 569-81.
115. Corey, E.J., C. Shih, and J.R. Cashman, *Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis.* Proc Natl Acad Sci U S A, 1983. 80(12): p. 3581-4.
116. Fischer, S., et al., *Uptake, release and metabolism of docosahexaenoic acid (DHA, c22:6 omega 3) in human platelets and neutrophils.* Biochem Biophys Res Commun, 1984. 120(3): p. 907-18.
117. Kim, H.Y., et al., *Stereochemical analysis of hydroxylated docosahexaenoates produced by human platelets and rat brain homogenate.* Prostaglandins, 1990. 40(5): p. 473-90.
118. Kim, H.Y., J.W. Karanian, and N. Salem, Jr., *Formation of 15-lipoxygenase product from docosahexaenoic acid (22:6w3) by human platelets.* Prostaglandins, 1990. 40(5): p. 539-49.
119. Hong, S., et al., *Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation.* J Biol Chem, 2003. 278(17): p. 14677-87.
120. Duffield, J.S., et al., *Resolvin D series and protectin D1 mitigate acute kidney injury.* J Immunol, 2006. 177(9): p. 5902-11.
121. Connor, K.M., et al., *Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis.* Nat Med, 2007. 13(7): p. 868-73.
122. Marcheselli, V.L., et al., *Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression.* J Biol Chem, 2003. 278(44): p. 43807-17.
123. Bazan, N.G., J.M. Calandria, and C.N. Serhan, *Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1.* J Lipid Res, 2010. 51(8): p. 2018-31.

124. Chen, P., et al., *Poxytrins, a class of oxygenated products from polyunsaturated fatty acids, potently inhibit blood platelet aggregation.* FASEB J, 2011. 25(1): p. 382-8.
125. Deng, B., et al., *Maresin biosynthesis and identification of maresin 2, a new anti-inflammatory and pro-resolving mediator from human macrophages.* PLoS One, 2014. 9(7): p. e102362.
126. Serhan, C.N., *Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways.* Annu Rev Immunol, 2007. 25: p. 101-37.
127. Hafstrom, I., et al., *Leukotriene B4--a stereospecific stimulator for release of lysosomal enzymes from neutrophils.* FEBS Lett, 1981. 130(1): p. 146-8.
128. Ringertz, B., et al., *Leukotriene-induced neutrophil aggregation in vitro.* FEBS Lett, 1982. 147(2): p. 180-2.
129. Camp, R.D., et al., *Responses of human skin to intradermal injection of leukotrienes C4, D4 and B4.* Br J Pharmacol, 1983. 80(3): p. 497-502.
130. Carbajal, V., et al., *LTD4 induces hyperresponsiveness to histamine in bovine airway smooth muscle: role of SR-ATPase Ca2+ pump and tyrosine kinase.* Am J Physiol Lung Cell Mol Physiol, 2005. 288(1): p. L84-92.
131. Foley, T.D., *5-HPETE is a potent inhibitor of neuronal Na+, K(+)-ATPase activity.* Biochem Biophys Res Commun, 1997. 235(2): p. 374-6.
132. Gordon, E.E., J.A. Gordon, and A.A. Spector, *HETEs and coronary artery endothelial cells: metabolic and functional interactions.* Am J Physiol, 1991. 261(4 Pt 1): p. C623-33.
133. Powell, W.S., D. Chung, and S. Gravel, *5-Oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration.* J Immunol, 1995. 154(8): p. 4123-32.
134. Chiang, N., M. Arita, and C.N. Serhan, *Anti-inflammatory circuitry: lipoxin, aspirin-triggered lipoxins and their receptor ALX.* Prostaglandins Leukot Essent Fatty Acids, 2005. 73(3-4): p. 163-77.
135. Maddox, J.F. and C.N. Serhan, *Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction.* J Exp Med, 1996. 183(1): p. 137-46.
136. Katoh, T., et al., *Renal hemodynamic actions of lipoxins in rats: a comparative physiological study.* Am J Physiol, 1992. 263(3 Pt 2): p. F436-42.
137. Nazarewicz, R.R., et al., *12(S)-hydroperoxyeicosatetraenoic acid (12-HETE) increases mitochondrial nitric oxide by increasing intramitochondrial calcium.* Arch Biochem Biophys, 2007. 468(1): p. 114-20.
138. Reilly, K.B., et al., *12/15-Lipoxygenase activity mediates inflammatory monocyte/endothelial interactions and atherosclerosis in vivo.* J Biol Chem, 2004. 279(10): p. 9440-50.
139. Armstrong, M.M., et al., *Inhibitory and mechanistic investigations of oxo-lipids with human lipoxygenase isozymes.* Bioorg Med Chem, 2014. 22(15): p. 4293-7.
140. Dho, S., et al., *Hepoxilin A3 induces changes in cytosolic calcium, intracellular pH and membrane potential in human neutrophils.* Biochem J, 1990. 266(1): p. 63-8.
141. Mrsny, R.J., et al., *Identification of hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia.* Proc Natl Acad Sci U S A, 2004. 101(19): p. 7421-6.

142. Matsuda, H., K. Miyatake, and S.E. Dahlen, *Pharmacodynamics of 15(S)-hydroperoxyeicosatetraenoic (15-HPETE) and 15(S)-hydroxyeicosatetraenoic acid (15-HETE) in isolated arteries from guinea pig, rabbit, rat and human.* J Pharmacol Exp Ther, 1995. 273(3): p. 1182-9.
143. Huang, J.T., et al., *Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase.* Nature, 1999. 400(6742): p. 378-82.
144. Takata, S., et al., *15-Hydroxyeicosatetraenoic acid inhibits neutrophil migration across cytokine-activated endothelium.* Am J Pathol, 1994. 145(3): p. 541-9.
145. Morris, T., et al., *Effects of low-dose aspirin on acute inflammatory responses in humans.* J Immunol, 2009. 183(3): p. 2089-96.
146. Maddox, J.F., et al., *Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor.* J Biol Chem, 1997. 272(11): p. 6972-8.
147. Buchanan, M.R., et al., *13-Hydroxyoctadecadienoic acid is the vessel wall chemorepellant factor, LOX.* J Biol Chem, 1985. 260(30): p. 16056-9.
148. Kogure, R., et al., *5-Hydroxy-eicosapentaenoic acid is an endogenous GPR119 agonist and enhances glucose-dependent insulin secretion.* Biochem Biophys Res Commun, 2011. 416(1-2): p. 58-63.
149. Strassburg, K., et al., *Quantitative profiling of oxylipins through comprehensive LC-MS/MS analysis: application in cardiac surgery.* Anal Bioanal Chem, 2012. 404(5): p. 1413-26.
150. Arita, M., et al., *Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1.* J Exp Med, 2005. 201(5): p. 713-22.
151. Bannenberg, G. and C.N. Serhan, *Specialized pro-resolving lipid mediators in the inflammatory response: An update.* Biochim Biophys Acta, 2010. 1801(12): p. 1260-73.
152. Sapieha, P., et al., *5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids.* Sci Transl Med, 2011. 3(69): p. 69ra12.
153. Li, R., et al., *Maresin 1, a Proresolving Lipid Mediator, Mitigates Carbon Tetrachloride-Induced Liver Injury in Mice.* Oxid Med Cell Longev, 2016. 2016: p. 9203716.
154. Gonzalez-Periz, A., et al., *Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA.* FASEB J, 2006. 20(14): p. 2537-9.
155. Kim, N., et al., *Specialized proresolving mediators (SPMs) inhibit human B-cell IgE production.* Eur J Immunol, 2016. 46(1): p. 81-91.
156. Schwab, J.M., et al., *Resolvin E1 and protectin D1 activate inflammation-resolution programmes.* Nature, 2007. 447(7146): p. 869-74.
157. Mukherjee, P.K., et al., *Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress.* Proc Natl Acad Sci U S A, 2004. 101(22): p. 8491-6.
158. Spector, A.A., *Arachidonic acid cytochrome P450 epoxygenase pathway.* J Lipid Res, 2009. 50 Suppl: p. S52-6.
159. Caligiuri, S.P., et al., *Flaxseed consumption reduces blood pressure in patients with hypertension by altering circulating oxylipins via an alpha-linolenic acid-induced inhibition of soluble epoxide hydrolase.* Hypertension, 2014. 64(1): p. 53-9.

160. Bylund, J., J. Ericsson, and E.H. Oliw, *Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography-mass spectrometry with ion trap MS.* Anal Biochem, 1998. 265(1): p. 55-68.
161. Bylund, J., et al., *Cytochromes P450 with bisallylic hydroxylation activity on arachidonic and linoleic acids studied with human recombinant enzymes and with human and rat liver microsomes.* J Pharmacol Exp Ther, 1998. 284(1): p. 51-60.
162. Oliw, E.H., J. Bylund, and C. Herman, *Bisallylic hydroxylation and epoxidation of polyunsaturated fatty acids by cytochrome P450.* Lipids, 1996. 31(10): p. 1003-21.
163. Shearer, G.C., et al., *Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters.* J Lipid Res, 2010. 51(8): p. 2074-81.
164. Westphal, C., A. Konkel, and W.H. Schunck, *CYP-eicosanoids--a new link between omega-3 fatty acids and cardiac disease?* Prostaglandins Other Lipid Mediat, 2011. 96(1-4): p. 99-108.
165. Agbor, L.N., et al., *Elevated blood pressure in cytochrome P4501A1 knockout mice is associated with reduced vasodilation to omega-3 polyunsaturated fatty acids.* Toxicol Appl Pharmacol, 2012. 264(3): p. 351-60.
166. Zhang, Y., et al., *EET homologs potently dilate coronary microvessels and activate BK(Ca) channels.* Am J Physiol Heart Circ Physiol, 2001. 280(6): p. H2430-40.
167. VanRollins, M., *Epoxygenase metabolites of docosahexaenoic and eicosapentaenoic acids inhibit platelet aggregation at concentrations below those affecting thromboxane synthesis.* J Pharmacol Exp Ther, 1995. 274(2): p. 798-804.
168. Ye, D., et al., *Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels.* J Pharmacol Exp Ther, 2002. 303(2): p. 768-76.
169. Carroll, M.A., et al., *Cytochrome P-450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides.* Am J Physiol, 1996. 271(4 Pt 2): p. R863-9.
170. Bednar, M.M., et al., *16(R)-hydroxyeicosatetraenoic acid, a novel cytochrome P450 product of arachidonic acid, suppresses activation of human polymorphonuclear leukocyte and reduces intracranial pressure in a rabbit model of thromboembolic stroke.* Neurosurgery, 2000. 47(6): p. 1410-8; discussion 1418-9.
171. Ishizuka, T., et al., *20-Hydroxyeicosatetraenoic acid stimulates nuclear factor-kappaB activation and the production of inflammatory cytokines in human endothelial cells.* J Pharmacol Exp Ther, 2008. 324(1): p. 103-10.
172. Randriamboavonjy, V., R. Busse, and I. Fleming, *20-HETE-induced contraction of small coronary arteries depends on the activation of Rho-kinase.* Hypertension, 2003. 41(3 Pt 2): p. 801-6.
173. McGiff, J.C. and J. Quilley, *20-HETE and the kidney: resolution of old problems and new beginnings.* Am J Physiol, 1999. 277(3 Pt 2): p. R607-23.
174. Node, K., et al., *Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids.* Science, 1999. 285(5431): p. 1276-9.
175. Yang, S., et al., *Cytochrome P-450 epoxygenases protect endothelial cells from apoptosis induced by tumor necrosis factor-alpha via MAPK and PI3K/Akt signaling pathways.* Am J Physiol Heart Circ Physiol, 2007. 293(1): p. H142-51.
176. Dhanasekaran, A., et al., *Multiple antiapoptotic targets of the PI3K/Akt survival pathway are activated by epoxyeicosatrienoic acids to protect cardiomyocytes from hypoxia/anoxia.* Am J Physiol Heart Circ Physiol, 2008. 294(2): p. H724-35.

177. Wang, Z., et al., *Arachidonic acid inhibits basolateral K channels in the cortical collecting duct via cytochrome P-450 epoxygenase-dependent metabolic pathways.* Am J Physiol Renal Physiol, 2008. 294(6): p. F1441-7.
178. Hercule, H.C., et al., *Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice.* Arterioscler Thromb Vasc Biol, 2009. 29(1): p. 54-60.
179. Oltman, C.L., et al., *Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation.* Circ Res, 1998. 83(9): p. 932-9.
180. Sakai, T., et al., *Leukotoxin, 9,10-epoxy-12-octadecenoate inhibits mitochondrial respiration of isolated perfused rat lung.* Am J Physiol, 1995. 269(3 Pt 1): p. L326-31.
181. Ozawa, T., et al., *Neutrophil microsomes biosynthesize linoleate epoxide (9,10-epoxy-12-octadecenoate), a biological active substance.* Biochem Biophys Res Commun, 1988. 152(3): p. 1310-8.
182. Nowak, G., D.F. Grant, and J.H. Moran, *Linoleic acid epoxide promotes the maintenance of mitochondrial function and active Na+ transport following hypoxia.* Toxicol Lett, 2004. 147(2): p. 161-75.
183. Siegfried, M.R., et al., *Direct cardiovascular actions of two metabolites of linoleic acid.* Life Sci, 1990. 46(6): p. 427-33.
184. Edin, M.L., et al., *Endothelial expression of human cytochrome P450 epoxygenase CYP2C8 increases susceptibility to ischemia-reperfusion injury in isolated mouse heart.* FASEB J, 2011. 25(10): p. 3436-47.
185. Moran, J.H., et al., *Cytotoxicity of linoleic acid diols to renal proximal tubular cells.* Toxicol Appl Pharmacol, 1997. 146(1): p. 53-9.
186. Zheng, J., et al., *Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome.* Am J Respir Cell Mol Biol, 2001. 25(4): p. 434-8.
187. Endo, J., et al., *18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload-induced maladaptive cardiac remodeling.* J Exp Med, 2014. 211(8): p. 1673-87.
188. Morin, C., et al., *Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung.* Am J Physiol Lung Cell Mol Physiol, 2009. 296(1): p. L130-9.
189. Morin, C., et al., *17,18-epoxyeicosatetraenoic acid targets PPARgamma and p38 mitogen-activated protein kinase to mediate its anti-inflammatory effects in the lung: role of soluble epoxide hydrolase.* Am J Respir Cell Mol Biol, 2010. 43(5): p. 564-75.
190. Jung, F., et al., *Effect of cytochrome P450-dependent epoxyeicosanoids on Ristocetin-induced thrombocyte aggregation.* Clin Hemorheol Microcirc, 2012. 52(2-4): p. 403-16.
191. Zhang, G., et al., *Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis.* Proc Natl Acad Sci U S A, 2013. 110(16): p. 6530-5.
192. Musiek, E.S., et al., *Cyclopentenone isoprostanes inhibit the inflammatory response in macrophages.* J Biol Chem, 2005. 280(42): p. 35562-70.
193. Liu, W., et al., *Ex vivo oxidation in tissue and plasma assays of hydroxyoctadecadienoates: Z,E/E,E stereoisomer ratios.* Chem Res Toxicol, 2010. 23(5): p. 986-95.
194. Spiteller, G., *Linoleic acid peroxidation--the dominant lipid peroxidation process in low density lipoprotein--and its relationship to chronic diseases.* Chem Phys Lipids, 1998. 95(2): p. 105-62.

195. Garscha, U., T. Nilsson, and E.H. Oliw, *Enantiomeric separation and analysis of unsaturated hydroperoxy fatty acids by chiral column chromatography-mass spectrometry.* J Chromatogr B Analyt Technol Biomed Life Sci, 2008. 872(1-2): p. 90-8.
196. Reich, E.E., et al., *Formation of novel D-ring and E-ring isoprostane-like compounds (D4/E4-neuroprostanes) in vivo from docosahexaenoic acid.* Biochemistry, 2000. 39(9): p. 2376-83.
197. Johnson, G.H. and K. Fritsche, *Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials.* J Acad Nutr Diet, 2012. 112(7): p. 1029-41, 1041 e1-15.
198. Yamaguchi, T., et al., *Dietary flax oil rich in alpha-linolenic acid reduces renal disease and oxylipin abnormalities, including formation of docosahexaenoic acid derived oxylipins in the CD1-pcy/pcy mouse model of nephronophthisis.* Prostaglandins Leukot Essent Fatty Acids, 2015. 94: p. 83-9.
199. Caligiuri, S.P., et al., *Dietary linoleic acid and alpha-linolenic acid differentially affect renal oxylipins and phospholipid fatty acids in diet-induced obese rats.* J Nutr, 2013. 143(9): p. 1421-31.
200. Caligiuri, S.P., et al., *Elevated levels of pro-inflammatory oxylipins in older subjects are normalized by flaxseed consumption.* Exp Gerontol, 2014. 59: p. 51-7.
201. Wang, Y., et al., *Comprehensive ultra-performance liquid chromatographic separation and mass spectrometric analysis of eicosanoid metabolites in human samples.* J Chromatogr A, 2014. 1359: p. 60-9.
202. Fischer, R., et al., *Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway.* J Lipid Res, 2014. 55(6): p. 1150-1164.
203. Keenan, A.H., et al., *Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers.* J Lipid Res, 2012. 53(8): p. 1662-9.
204. Schebb, N.H., et al., *Comparison of the effects of long-chain omega-3 fatty acid supplementation on plasma levels of free and esterified oxylipins.* Prostaglandins Other Lipid Mediat, 2014. 113-115: p. 21-9.
205. Metherel, A.H., et al., *Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women.* Prostaglandins Leukot Essent Fatty Acids, 2009. 81(1): p. 23-9.
206. Schuchardt, J.P., et al., *Modulation of blood oxylipin levels by long-chain omega-3 fatty acid supplementation in hyper- and normolipidemic men.* Prostaglandins Leukot Essent Fatty Acids, 2014. 90(2-3): p. 27-37.
207. Zulyniak, M.A., et al., *Fish oil supplementation alters circulating eicosanoid concentrations in young healthy men.* Metabolism, 2013. 62(8): p. 1107-13.
208. *Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA); Panel on Dietetic Products, Nutrition and Allergies*. 2012, European Food Safety Authority: EFSA Journal. p. 2815.
209. Bakewell, L., G.C. Burdge, and P.C. Calder, *Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets.* Br J Nutr, 2006. 96(1): p. 93-9.
210. Kitson, A.P., C.K. Stroud, and K.D. Stark, *Elevated production of docosahexaenoic acid in females: potential molecular mechanisms.* Lipids, 2010. 45(3): p. 209-24.

211. Manassiev, N. and M.I. Whitehead, *Female reproductive health*. 2004, New York: Parthenon Pub. Group. vii, 195 p.
212. Booth, E.A., et al., *Estrogen protects the heart from ischemia-reperfusion injury via COX-2-derived PGI2.* J Cardiovasc Pharmacol, 2008. 52(3): p. 228-35.

1.5 Study Rationale

After examining the literature it is obvious that a number of clear gaps exist. No one has ever directly compared the supplementation of ALA rich plant and DHA rich marine oils at 4 g/day, over a time course (1, 3, 7, 17 and 28 days) on the oxylipin profiles of young (19-35), healthy males and females. There is specific interest to understand the effects of omega-3 fatty acids and the differences between ALA and DHA. A novel DHA rich fish oil will help us to further understand the impact of DHA supplementation on the oxylipin profile. Further, no one has examined the impact of ALA supplementation from flax oil on the oxylipin profiles of young individuals.

Due to differences in fatty acid metabolism between sexes, it is critical that we examine the differences that may exist between young males and females to better understand how omega-3 supplementation impacts the body. No one has examined if the oxylipin profile is altered by the female menstrual cycle during periods of omega-3 supplementation and this information could further help us to understand the impact of dietary lipids. Obtaining information about the oxylipin profiles of healthy young individuals being supplemented with omega-3 fatty acids will also provide a comparison for older individuals or those experiencing disease conditions.

1.6 Hypothesis

ALA (4 g/day) and DHA (4 g/day) will have different effects on the oxylipin profiles. Further, males will display different oxylipin profiles from females who are consuming the same oil treatment. The ALA oil group will experience an increase in ALA, DHA, and EPA derived oxylipins from baseline due to conversion and the DHA group will experience an increase in DHA and EPA derived oxylipins. Further, there will be rapid conversion (within 1 day) to

oxylipins in both sexes consuming DHA enriched fish oil. Differences between plasma and serum oxylipins will be present between oxylipins involved in blood clotting. Supplemental oils will have small quantities of oxylipins within them, ALA derived oxylipins in flax oil and DHA and EPA derived oxylipins in fish oil.

Objectives 1-4 will be covered in Chapter 2: ALA and DHA Rich Oils Alter Blood Oxylipin Profiles Differently in Young Healthy Males and Females.

1.7 Objectives

1) Determine how ALA and DHA at 4 g/day impact the oxylipin profile over a time course (1, 3, 7, 14, and 28 days).
2) Determine if sex causes a change in the oxylipin profiles of persons supplemented with ALA or DHA at 4 g/day.
3) Determine if sex causes differences in plasma compared to serum oxylipins after 28 days of ALA or DHA treatment.
4) Determine the oxylipin profiles of both ALA and DHA supplemental oils.

2 CHAPTER 2 - ALA and DHA Rich Oils Alter Blood Oxylipin Profiles Differently in Healthy Young Male and Females.

Melissa Gabbs, Carla G. Taylor, Peter Zahradka, Tanja Winter, Harold M. Aukema

University of Manitoba, Department of Human Nutritional Sciences, University of Manitoba Department of Physiology and Pathophysiology, Canadian Center for Agri-Food Research in Health and Medicine, St. Boniface Hospital

Contribution of co-authors to Chapter 2:

Melissa Gabbs – Recruited participants, conducted study visits (measured blood pressure, processed blood samples), performed fatty acid analysis (plasma, serum, and investigational oils), extracted, analyzed, and quantified oxylipins (plasma, serum, and investigational oils), performed all statistical analysis. Wrote Chapter 2 and created all tables and figures.

Tanja Winter – Maintained and ran samples on HPLC-MS/MS, calculated detector response factors for all oxylipins, calculated amount of internal standard to be added to each sample, assisted with questions and education regarding HPLC-MS/MS

Carla Taylor – Submitted for ethics approval, developed study design.

Peter Zahradka – Developed study design, randomized samples for distribution

Harold Aukema – Developed study design.

Keywords: Oxylipins, Alpha-linolenic acid, Lipidomics, Docosahexaenoic acid

2.1 Abstract

Background: Time course changes in oxylipin profiles among healthy, young males and females consuming high doses of ALA compared to DHA (4 g/day) remain to be determined. Due to differences in lipid metabolism between sexes, it is important that males and females are examined separately when studying the effect of omega-3 supplementation on the oxylipin profile.

Methods: Young, healthy individuals were recruited to participate in a double blind randomized cross over trial. Participants (n=12) consumed ALA oil (4 g/day ALA) and DHA oil (4 g/day DHA + 0.8 g/day EPA) in capsule format for 4 weeks. Clinic visits were held at day 0, 1, 3, 7, 14, and 28. Solid phase extraction was used to isolate oxylipins followed by analysis using HPLC MS/MS and quantification utilizing stable isotope dilution.

Results: DHA oxylipins increased after as little as one day of DHA oil supplementation, with females responding more quickly and achieving higher levels than males. Supplementation of ALA at 4 g/day increased plasma ALA and a few individual ALA oxylipins but did not increase all ALA derived oxylipins. Oxylipins from several PUFA precursors were present in greater quantities in serum and females had higher levels of several LA and DHA oxylipins whereas males had higher levels of several AA COX oxylipins. Oxylipins in high concentrations were also found in both the ALA and DHA rich oils.

Conclusion: DHA oil increased DHA derived oxylipins in both sexes, with alterations occurring more quickly in females, whereas few alterations in oxylipin profiles occurred in response to ALA oil.

2.2 Introduction

Omega-3 fatty acids vary in structure and function and can be generated by plants and certain animals. Plant derived omega-3 fatty acids (e.g. ALA) are commonly found in flax and flax oil products, while marine-derived omega-3 fatty acids (e.g. EPA and DHA) are found in fatty fish and shellfish.

ALA is enzymatically converted to EPA and DHA; however, this process occurs at a low rate and differs between sexes, where females convert ALA to EPA and DHA at higher rates than males [1]. It has also been observed that females have higher circulating DHA levels than males, likely due to the effect of estrogen [2]. Post-menopausal women also display differences in fatty acid profiles when compared to males, likely due to decreased estrogen [3]. Due to this possible estrogen effect it is necessary to take age and sex into consideration when examining dietary lipids.

Omega-3 fatty acid supplementation, particularly from marine oils, has become popular in recent years due to the perceived benefits reported in the scientific literature. Meta-analyses reveal long chain omega-3 PUFAs may decrease the risk of cardiovascular disease [4, 5] and cardiac death [6] while plant based ALA may also have a potential beneficial role in lowering CVD risk [7]. The oxygenated metabolites produced from PUFA may be one way they exert their functions in the body.

Oxylipins are oxygenated metabolites of PUFA, many of which are thought to be involved in paracrine signaling. These oxygenated metabolites are produced through three main pathways: COX, LOX, and cP450 and produce a profile in plasma and serum that changes in response to diet. Our knowledge regarding oxylipins was previously limited to the products of the well-known 20-carbon fatty acids (eicosanoids), namely arachidonic acid, which produced oxylipins

that have critical roles in homeostasis. Due to recent advances in HPLC-MS/MS technologies we are now able to identify and quantify oxylipins that are produced from several other 18-, 20-, and 22-carbon PUFA parents including ALA, EPA, and DHA. See the review by Gabbs *et al.* [8] for a comprehensive review of the production and function of PUFA derived oxylipins (Appendix 4.1).

Oxylipin profiles are reflective of the amount of available PUFA substrate, which means fatty acid consumption and duration of consumption could all impact the profile. Supplementing fish oil of different compositions to the diet has been shown to alter oxylipin profiles among a number of different population groups [9-12], while providing ALA in the form of flax oil and examining the resultant oxylipin profiles in humans has not been seen in the literature.

Previous studies examining oxylipin profiles in response to omega-3 supplementation gave various doses, ratios, and sources of EPA, and DHA to participants. Fish oil supplements have been examined are approximately equal concentrations of EPA and DHA [13], while others provided higher ratios of EPA: DHA [9-11]. We lack data that examines the impact of DHA rich fish oil on the oxylipin profile in humans. The average amount of EPA plus DHA present in one portion of oily fish is 3.27 g/day [14] and few studies have examined supplementation higher than this average [9, 10] in relation to the oxylipin profile. With an upper limit of 5 g/day of total EPA and DHA deemed safe to consume by the European Food Safety Authority [15], it is important to have a good understanding of how supplementation at all doses, including high doses, impacts the oxylipin profile. Finally, previous studies have provided a partial understanding of how several weeks of fish oil supplementation impacts the oxylipin profile [9, 10] and also how a single dose of fish oil impacts oxylipin production [11]. However, we still lack an understanding of how the oxylipin profile responds to supplementation over time.

Supplementation studies investigating the effects of ALA on health often provide ALA in various forms to participants (i.e. flax oil, milled flax seed) and in various doses, however, most of these studies have looked exclusively at fatty acids and not oxylipins [7]. Having some clarity of how flax oil impacts oxylipin profiles at a dose of 4 g/day, which is higher than the Adequate Intake of 1.1 g/day for females and 1.6 g/day for males [16], would help us to better understand the impact of supplementation in the body. ALA supplementation from a flax seed muffin (6 g/day) for both 4 weeks and 6 months both showed changes in oxylipins derived from several PUFA [17, 18]. Further, the effect of ALA rich fish oil and DHA rich flax oil on the oxylipin profile have never been directly compared.

Due to differences in fatty acid metabolism between sexes, previously reported oxylipin responses to supplementation may not be reflective of pre-menopausal women. Previous studies have not included females [11, 13] or included females of varying ages [12, 19, 20], thereby not taking menopause into account. Caligiuri *et al.* examined several differences in the oxylipin profiles of older compared to younger individuals including increase in several pro-inflammatory LA and AA derived oxylipins [18]. Further, females generally display higher baseline plasma DHA [2], suggesting it may be important to examine changes in oxylipin profiles within defined groups of individuals as opposed to looking at a whole population. Information is lacking regarding how this and other sex differences in lipid metabolism impact how the body responds to omega-3 PUFA supplementation. It also is not known if males and females respond to omega-3 supplementation by producing oxylipins at the same rate. Additionally, these two groups (ALA versus DHA) have not been compared and due to their relationship along the conversion pathway it would be beneficial to examine the role supplementation has on the oxygenated metabolites produced from these two omega-3 fatty acids.

Much is also still unknown regarding oxylipins and their production, particularly if differences exist between plasma and serum oxylipin profiles. Several papers have aimed to address what these profiles look like in healthy humans, however, their analysis combines sexes and pre- and post-menopausal women [21, 22]. Examining differences between plasma and serum profiles after omega-3 supplementation will help us to better understand the impact supplementation has on these two tissues and which may be more advantageous for oxylipin analysis. Finally, the oxylipin content of these investigational oils has not been examined before. This is important for understanding exactly what is found in these nutritional supplements and what might be contributing to their physiological effects, benefiting both basic and clinical studies in the future.

In this study we provide ALA rich flax oil and DHA rich fish oil at a 4 g/day dose to a young population and examine alterations in oxylipin profiles over time so that we can better understand how supplementation differentially impacts males and females. We also analyzed oxylipins present in the investigational omega-3 oils to further understand our treatment oils.

2.3 Experimental Procedures

Participants for this double blind randomized control trial were recruited from the Winnipeg, Manitoba area using print advertisements. Participants were first screened to determine eligibility for the study and all participants gave written informed consent before they were accepted into the study. This study was approved by the University of Manitoba Research Ethics Board and St. Boniface Hospital Research Review Committee.

Subjects

Participants were pre-screened and excluded from participation if they had a clinically diagnosed disease currently affecting the circulatory, respiratory, immune, skeletal, urinary,

muscular, endocrine, digestive, nervous or reproductive system, or a disease condition that had required medical treatment. Additional exclusion criteria included regular use of non-steroidal anti-inflammatory drugs (NSAID) or omega-3 supplements within the past 3 months, allergy or sensitivity to fish or flax products, current or past cigar/cigarette smoking, unstable body weight in the past 6 months (±3kg), consumption of >15 alcoholic beverages/week within the past 3 months or while participating in the study, current viral, bacterial, fungal infection, or donation of blood in the past 2 months.

Twelve individuals (6 male, 6 non-pregnant, non-lactating female) were selected between 18-50 with a body mass index (BMI) between 18-28 and blood pressure (BP) <140/90 were selected for the study. These individuals also displayed normal blood lipid profiles, plasma creatinine (<1.5x upper limit, where normal is 50-97 umol/L), liver enzymes (aspartate transaminase: <2x upper limit, where normal is 10-32 U/L; alanine transaminase <2x upper limit where normal is <25U/L) and normal menses if female; and were willing to follow the study protocol. Actual study population parameters are listed in Table 2-1.

Table 2-1: Study population baseline parameters.
Minimum and maximum values listed with total population means. Values are reported as mean±standard error (SEM).

Parameter	**Total Population** (n=12) **mean±SEM**	**Males** (n=6) **mean±SEM**	**Females** (n=6) **mean±SEM**	**Minimum**	**Maximum**
Age (years)	25 ± 1	25 ± 1	25 ± 1	19	34
Body height (cm)	172 ± 2	179 ± 3	165 ± 2	155	188
Body weight (kg)	73 ± 2.4	76 ± 4.4	70 ± 1.8	57	98
BMI (kg/m^2)	24.6 ± 0.51	23.5 ± 0.81	25.7 ± 0.49	20.6	27.8
Systolic BP (mmHg)	114 ± 2	115 ± 3	114 ± 3	104	126
Diastolic BP (mmHg)	76 ± 1	75 ± 2	77 ± 2	68	88

Study Design

Accepted participants were subjected to a minimum 6 week wash-in period where they were instructed to avoid consumption of foods high in ALA, EPA, and DHA (defined as >0.3 grams ALA/serving, or >0.1 grams EPA + DHA/serving), and which was followed for the duration of the study. Following the wash-in period, participants were subjected to a 4-week supplementation period; they then completed a minimum 6-week wash-out period followed by a second 4-week supplementation period. Due to the nature of the cross-over design the participants were randomly assigned to one of the treatments during the first supplementation phase and then assigned to the other treatment during their second supplementation phase (Figure 2-1). The wash-in and wash-out periods varied in length to ensure females to began the supplementation period 9 ± 2 days after the beginning of menses.

At day 0 of each treatment phase, participants attended the clinic after an overnight fast for baseline testing. Height, weight and BP were measured and blood was collected. On days 1, 3, 7, 14, and 28 participants also came into the clinic fasted and provided a blood sample. Weight and BPP was also measured at day 28 of each supplementation phase. After completion of the day 28 visit participants entered their minimum 6-week wash-out period - or if this was their second supplementation phase, completed the study.

Completion of activity questionnaires and 3-day food records helped to monitor participant compliance and determine background diet and activity levels. Plasma fatty acid composition was also examined to help determine participant compliance.

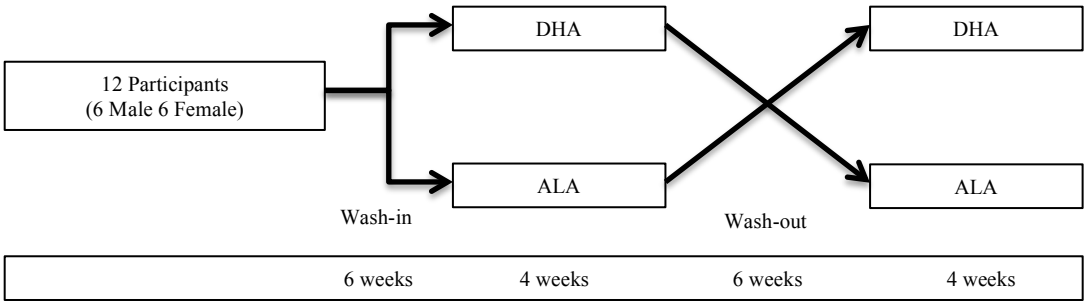


Figure 2-1: Graphical representation of study design.
In this double blind randomized cross over trial, 12 participants were included to consume both ALA and DHA treatments, each for four weeks. Minimum six-week wash in and wash out periods were included prior to beginning the study and between treatments.

Investigational Product

Participants were provided with a 2-week supply of study capsules as well as directions for consumption at their day 0 clinic visit. Participants were instructed to consume flax oil capsules that contained 4 g/day of ALA (NOW flax oil capsules from Now Foods 60108. Imported and distributed by Puresource Inc, Guelph, ON N1H 6J4. NPN:80002313) or DHA (+0.8 grams/day EPA) in capsule form (Super DHA gems manufactured for J.R. Carlson Laboratories, Arlington Heights, IL. 60004-1985. Imported by MMP Enterprises, Concord, ON L4K 5W2. NPN: 80012587). Flax oil capsules (1000 mg) contained 550 mg of ALA and fish oil capsules (1000 mg) contained 500 mg of DHA and 100 mg of EPA as per manufactures specifications. Fatty acid analysis of both the ALA and DHA oils was performed and our results confirm this composition (results are presented in Appendix 4.7 **Table 0-4**). Subjects were instructed to ingest 7 capsules per day on the flax oil treatment or 8 capsules per day on the fish oil treatment. Capsules were prepackaged by a pharmacy (Tache Pharmacy Winnipeg, MB R2H 3C3) into 3 or 4 capsules per pack with 2 packs designated per day and put into blinded packaging. Participants were asked to consume the packets with water, one with a morning meal and the second with an afternoon or evening meal. As per manufacturer specifications, daily intake was 3850 mg/day of ALA from flax oil or 4000 mg/day DHA (+ 800 mg EPA) from fish oil. Participants were provided with a second 2-week supply of study capsules at their day 14 visit and instructed to bring back any missed capsule doses to their day 14 and day 28 visits. From this point onwards the groups consuming 4 g/day of ALA from flax oil and 4 g/day DHA from fish oil will be referred to as ALA and DHA oil groups respectively.

Sample Collection and Clinical Parameter Analysis

Blood samples were collected from participants after completing an overnight fast via venipuncture of an arm vein by a Registered Nurse or trained phlebotomist and immediately processed and frozen at -80^{0}C until further analysis was done. Samples collected on day 0 and day 28 study visits were drawn into one 10.0 mL ethylenediamine tetraacetic acid (EDTA), one 4.0 mL EDTA, one 4.5 mL lithium heparin (LiHEP), and two 5.0 mL serum separator tube (SST) tubes [BD Vacutainer Blood Collection Tubes (Becton Dickinson, Heidelberg, Germany)]. Blood samples collected from days 1, 3, 7, and 14 were drawn into one 10.0 mL EDTA and one 4.0 mL EDTA tubes. Samples from days 0 and 28 drawn into LiHEP tubes were sent to St. Boniface Hospital Biochemistry Department for blood lipid analysis.

For plasma preparation, blood samples were centrifuged at 1100 g at 4^{0}C; supernatants were then aliquoted and frozen at -80^{0}C until further analysis was completed. For serum preparation, blood samples were incubated at room temperature for 30-45 minutes then centrifuged at 1170 g. Serum was aliquoted and frozen at -80^{0}C until further analysis was completed. All plasma and serum samples for fatty acid and oxylipin analysis had antioxidant cocktail (0.2 mg/mL butylated hydroxytoluene (BHT), 0.2 mg/mL EDTA, 2 mg/mL triphenylphosphine (TPP), 2 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) added at 3.3% of sample volume. A detailed protocol is given in Appendix 4.2.

Analysis of Fatty Acids and Oxylipins

Lipids were extracted from plasma, methylated, and quantified using gas chromatography (GC). Briefly, 2.5 mL 2:1 chloroform:methanol with 0.01% BHT (0.03 g BHT, 200 mL chloroform, 100mL methanol) was added to 250 µL plasma. Next, 100 µL of the 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (1.8 mg/mL in chloroform) was added to each sample followed by 2.25 mL of chloroform:methanol, and 950 µL 0.73% sodium chloride.

Samples were centrifuged for 10 minutes at 800 g and the lower phase of the biphasic mixture was transferred into a new glass vial. Next, samples were dried down in a nitrogen evaporator water bath at 37^0C and reconstituted with 1.2 mL methanolic sulfuric acid (6.0 mL sulfuric acid, 94 mL methanol). Samples were tightly capped and placed in an 88^0C oven for 1.5 hours. Next, 1.5 mL toluene and 1.0 mL ultrapure water were added and samples were centrifuged at 800 g for 5 minutes. The top layer of the biphasic mixture was transferred to a clean 2.0 mL GC vial. Samples were then dried down in a nitrogen evaporator water bath at 37^0C and reconstituted with 50 µL hexane. Samples were stored at -20^0C until analyzed on GC. Samples were separated on a DB225MS column (30 m X 0.25 mm diameter and 0.25 mm film thickness; Agilent Technologies Canada Inc., Mississauga, Ontario) using a 450-GC with a flame ionization detector and hydrogen as a carrier gas. Total run time was 46:40 minutes and 20:1 split ratio was used with a column flow of 1.3 mL/min. Fatty acids were quantified using internal standard and values are expressed as nmol/L of plasma. Step by step details are listed in Appendix 4.3.

Lipids were extracted from investigational oil capsules, methylated, and analyzed by GC. All lipids were extracted from capsules into hexane and then dried down under a nitrogen evaporated water bath at 37^0C leaving only the lipid contents of the capsule. Briefly, 1.0 mL of hexane was added to 0.11 g of oil. Next, 10 µL of internal standard heptadecanoic acid internal standard [(5.5 mg/mL in chloroform) Nu Check Prep Inc., catalogue no. U-17-A] was added to each sample, vortexed, and 100 µL was transferred to a new tube and dried down in a nitrogen evaporator water bath at 37^0C. Next, samples were reconstituted of 1.0 mL of toluene and 1.2 mL methanolic sulfuric acid (6.0 mL sulfuric acid, 94.0 mL methanol). Samples were then tightly capped and placed in an 80^0C oven for 60 minutes. Next, 1.0 mL ultrapure water and 1.0 mL hexane were added to the samples, vortexed, and centrifuged at 800 g. The top layer of the

samples was transferred to a new glass tube and 2.0 mL of ultra pure water was added, vortexed and centrifuged at 800 g for 5 minutes. Approximately 1.0 mL of the top phase of the biphasic mixture was transferred to a clean 2.0 mL GC vial. Samples were stored at -20^{0}C until analyzed by GC.

Samples were separated on a DB225MS column (30 m X 0.25 mm diameter and 0.25 mm film thickness; Agilent Technologies Canada Inc., Mississauga, Ontario) using a 450-GC with a flame ionization detector and hydrogen as a carrier gas. Total run time was 46:40 minutes and 20:1 split ratio was used with a column flow of 1.3 mL/min. Fatty acids were quantified using internal standard and values are expressed as % fatty acid per capsule. Step by step details are listed in Appendix 4.4.

For plasma and serum oxylipin analysis, deuterated internal standards (Cayman Chemical) were added to a 400 µL aliquot of prepared serum or plasma. Samples were first acidified to pH3 with 1N HCl and centrifuged. Strata-X (30u, 60mg/3mL) solid phase extraction columns (Phenomenex) were pre-conditioned with 2.0 mL methanol followed by 2.0 mL pH3 water. Supernatant was applied to column and allowed to pass through by gravity. A 10% methanol in pH3 water wash was applied to the tube as a wash, centrifuged and the supernatant was added to the column. Samples were eluted from the column with 1.0 mL of 100% methanol. When ready for HPLC, MS/MS samples were dried down in a nitrogen evaporator water bath at 37^{0}C and reconstituted with 100 µL solvent A (water:acetonitrile:formic acid (70:30:0.02 v/v/v)).

Oxylipins in oil capsules were also separated with HPLC-MS/MS. Briefly, 1350 µL of 100:1:2 methanol:formic acid:antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2mg/mL TPP, 2 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:H_2O) was added to 0.02 g of oil. Samples were vortexed and 750 µL was added to 3750 µL pH3 water, and 130 µL

of deuterated internal standard (Cayman Chemical). Samples were then acidified to pH3 with 1N HCl. Strata-X (30 u, 60 mg/3mL) solid phase extraction columns (Phenomenex) were pre-conditioned with 3.5 mL methanol followed by 3.5 mL pH3 water. The sample was applied to the column and allowed to pass through by gravity. A 10% methanol in pH3 water wash was applied to the sample tube and added to the column after pelleting debris by centrifugation. The column was then rinsed with 2.0 mL pH3 water and 1.0 mL hexane and pushed through to dry. Samples were eluted from the column with 1.0 mL of 100% methanol. When ready for HPLC MS/MS samples were dried down in a nitrogen evaporator water bath at 37^0C and reconstituted with 1.3 mL solvent A (water:acetonitrile:formic acid (70:30:0.02 v/v/v).

Oxylipins in plasma, serum, and investigational oils were separated with HPLC-MS/MS using a Luna 5µm C18(2) 100Å 250 x 2.0 mm column on a Shimadzu Nexera XR HPLC and ABSciex QTRAP 6500 MS with triple quadrapole electrospray ionization (IonDrive Turbo V) with multiple reaction monitoring. An injection volume of 40 µL was used and a flow rate of 0.3 mL/min. Detector response factors were calculated and used to quantitate oxylipins (Appendix 4.6 **Table 0-2**). Oxylipins are expressed as pM of plasma or serum or mM of analyte concentration in oil capsule. Step by step details are listed in Appendix 4.5 and 4.6.

Statistical Analysis

All data were analyzed using SAS University Edition (SAS Institute Inc., Cary, NC) and data are presented as mean±SEM where $p< 0.05$ is considered significant. Anthropometric, blood lipid, fatty acid, and plasma oxylipin data were analyzed using mixed modeling for repeated measures where comparison of time points to baseline, treatments and sexes at time points, and overall sex, treatment, and time effects were determined.

In analyzing repeated measures data, individual differences in changes over time are typically captured by random effects using mixed modeling (the multilevel model for change). These random effects describe individual trends across time, and explain the correlational structure of the longitudinal data. For individual trend over time, we used quadratic model to estimate both linear and quadratic slopes. Then those slopes were used to calculate the plateau. The approach used for the parameter estimation in mixed model is maximum likelihood method.

Comparison of serum and plasma oxylipin data were first analyzed for normality using the Shapiro-Wilk Test. Data were analyzed using three-way ANOVA followed by a post-hoc comparison using Tukey's Studentized Range Test. Non-Gaussian data were analyzed by Kruskal-Wallis prior to post-hoc comparison, also using Tukey's Studentized Range Test.

2.4 Results

All but one of the 12 participants completed the study (removed midway through the second supplementation phase due to conflicting schedule). Capsule consumption compliance was 93% as per participant reporting and fatty acid analysis revealed that all individuals were within two standard deviations of the mean for both ALA and DHA at all time points, suggesting good compliance among participants. Self reported 3-day food records and activity questionnaires were collected from participants, but only minor differences were observed (i.e. riboflavin in ALA oil treatment increased, and alterations in the way total walking time per week was reported) and these are listed in Appendix 4.7 Table 0-12 and Table 0-13.

Clinical Parameters

DHA from fish oil at 4 g/day and ALA from flax oil at 4 g/day had no treatment or time effect on weight, BMI, or systolic/diastolic BP of participants. Additionally, time or treatment had no effect on the participant's cholesterol, triglycerides, HDL, LDL, TC (total

Table 2-2. Participant fasting blood lipid levels and anthropometrics at baseline and day 28 for ALA and DHA oil treatments. *No treatment or time effects were observed. Sex effect indicates differences between sexes regardless of treatment or time. Values represent the mean±SEM.*

	ALA				DHA			
	Day 0		Day 28		Day 0		Day 28	
	Male	Female	Male	Female	Male	Female	Male	Female
BMI	23.6 ± 1.1	25.5 ± 0.7	24.0 ± 1.2	25.9 ± 0.8	23.8 ± 1.1	25.9 ± 0.7	23.6 ± 1.1	25.8 ± 0.7
Systolic BP	118 ± 2.8	114 ± 4.4	118 ± 3.5	112 ± 5.3	119 ±2.6	119 ± 4.4	117 ± 6.6	113 ± 4.6
Diastolic BP	77 ± 2.0	76 ± 2.8	74 ± 3.4	74 ± 3.1	75 ± 1.2	79 ± 2.6	74 ± 2.8	76 ± 2.7
TC (mmol/L)	4.2 ± 0.1	4.7 ± 0.3	4.5 ± 0.2	4.5 ± 0.4	4.3 ± 0.1	4.4 ± 0.3	4.6 ± 0.3	4.4 ± 0.2
TAG (mmol/L)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.3	0.7 ± 0.0	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
HDL (mmol/L)[1]	1.4 ± 0.1	2.1 ± 0.3	1.4 ± 0.1	2.3 ± 0.4	1.3 ± 0.1	2.1 ± 0.3	1.4 ± 0.1	2.1 ± 0.4
LDL (mmol/L)[1]	2.4 ± 0.2	2.2 ± 0.2	2.7 ± 0.2	2.0 ± 0.1	2.6 ± 0.2	1.9 ± 0.2	2.7 ± 0.3	1.8 ± 0.2
TC/HDL[1]	3.3 ± 0.3	2.4 ± 0.2	3.4 ± 0.3	2.2 ± 0.3	3.5 ± 0.3	2.3 ± 0.2	3.3 ± 0.3	2.4 ± 0.3
LDL/HDL[1]	1.9 ± 0.2	1.2 ± 0.2	2.1 ± 0.3	1.1 ± 0.2	2.1 ± 0.3	1.1 ± 0.2	2.0 ± 0.3	1.1 ± 0.3

[1]Sex Effect

cholesterol)/HDL, or LDL/HDL levels. An overall sex effect was observed where females had higher HDL and lower LDL, TC/HDL, and LDL/HDL regardless of time point or treatment. Blood lipid and anthropometric results can be found in **Table 2-2**.

Plasma Fatty Acids

With the sexes together: C18:1ω7(cis), C18:2ω6, C183ω6, and C20:0 were different between treatments at baseline, all were more elevated in the DHA oil group compared to the ALA oil group. When sexes are separated, no differences were observed for any plasma fatty acid at baseline between males and females for either treatment. Average baseline plasma ALA, EPA, and DHA with the sexes combined were 74 nmol/L, 84 nmol/L, and 160 nmol/L, respectively. No differences between treatments or sexes at baseline were observed for these fatty acids suggesting washout and wash-in periods were of sufficient length.

Both ALA and DHA oil treatments caused specific changes in several plasma fatty acids between baseline and day 28 when the sexes are combined. In response to ALA oil treatment: plasma ALA, EPA, DPAω3, and total omega-3 fatty acids were elevated from baseline at day 28 (Appendix 4.7 **Table 0-5**). No changes were observed for plasma DHA. In response to DHA oil treatment: EPA, DHA, and total omega-3 fatty acids were elevated from baseline at day 28 (Appendix 4.7 **Table 0-5**). No increases were observed in response to DHA oil treatment for ALA or DPAω3. Further alterations were observed with omega-6 plasma fatty acids whereas ALA and DHA oils had opposite effects at day 28 with ALA oil increasing total omega-6 fatty acids and DHA oil decreasing several specific fatty acids as well as total omega-6 fatty acids (Appendix 4.7 **Table 0-5**).

Alterations in plasma fatty acids occur both immediately and later in the time course and vary with treatment when the sexes are combined. ALA in response to ALA oil treatment and EPA and DHA in response to DHA oil treatment become elevated at day 1 and remain elevated throughout the treatment period (Appendix 4.7 **Table 0-5**). Total omega-3 fatty acids were immediately elevated in response to DHA oil and remained elevated throughout the treatment where as ALA oil increased total omega-3 fatty acids beginning at day 7 and throughout the remainder of the study (Appendix 4.7 **Table 0-5**). In response to DHA oil treatment, total omega-6 fatty acids were decreased from baseline beginning at day 3 where as total omega-6 fatty acids were increased at day 7 and 28 in response to ALA treatment (Appendix 4.7 **Table 0-5**). DPAω6, in response to DHA oil treatment, did not follow the same decreasing trend as the other omega-6 fatty acids as it was elevated at days 1, 3, 7, and 28. Finally, total PUFA was increased with ALA oil treatment at day 7 and 28 and not altered with DHA oil treatment.

Differences in plasma fatty acids in males and females were more often observed in response to ALA oil treatment; however, some differences were also observed in response to DHA oil treatment. Females displayed higher ALA from baseline in response to ALA oil treatment from day 1 onwards whereas males displayed elevated ALA from baseline at day 7 onwards (Appendix 4.7 **Table 0-6**, **Figure 2-2**). This difference is also reflected in calculated plateaus for ALA, where females reached a plateau at day 5 and males at day 6 of ALA oil treatment (Appendix 4.7 **Table 0-14**). Further, females displayed higher plasma EPA from baseline at day 7 and 28 and higher than males at day 28, while males displayed no differences from baseline (**Figure 2-2**). In response to DHA oil treatment, both males and females displayed higher EPA and DHA from baseline at day 1 onwards. However, sex differences were seen when calculating the time at which a plateau was reached: females reached a plateau at 5 days for EPA

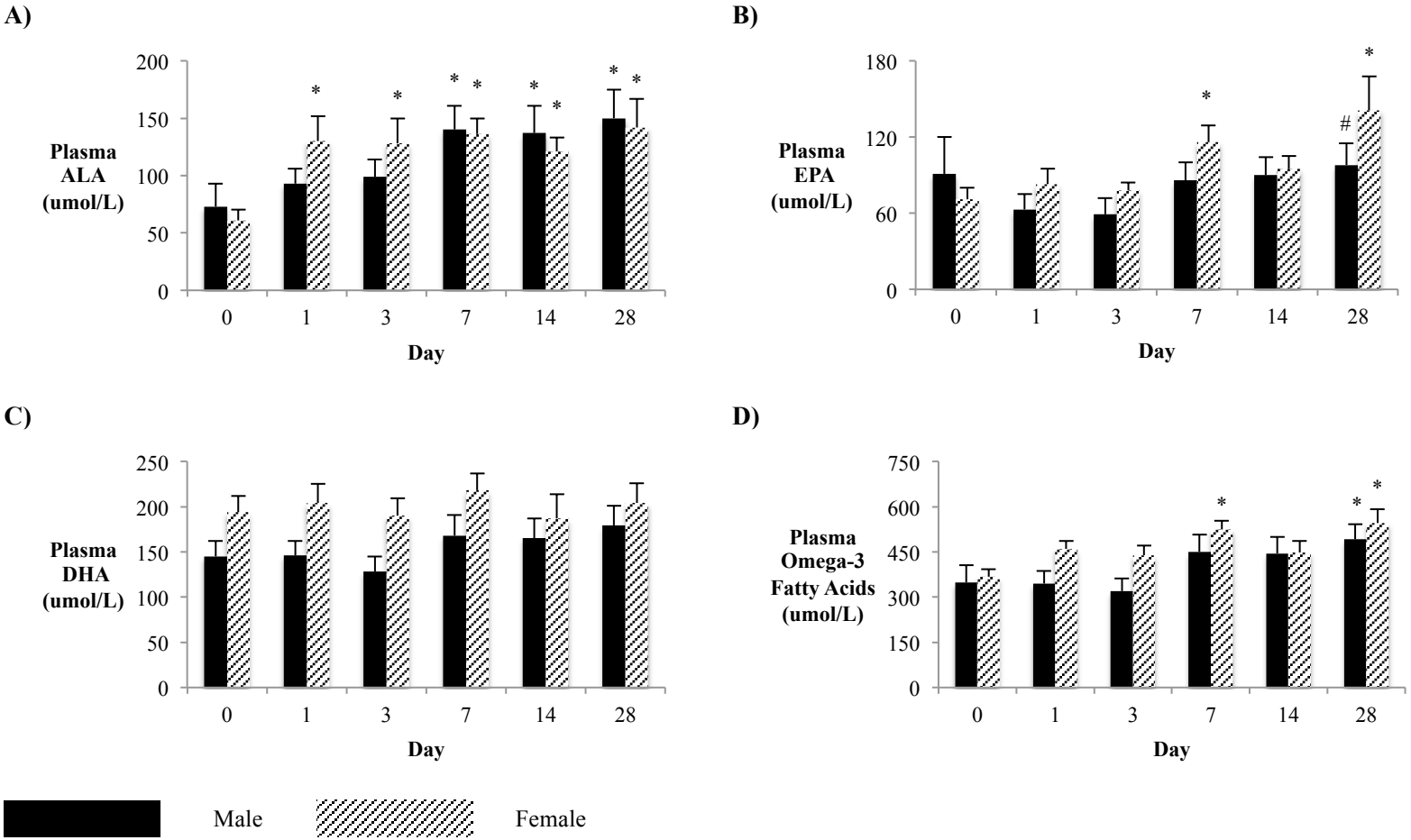


Figure 2-2. Select omega-3 plasma fatty acids among healthy individuals consuming ALA oil.

Select omega-3 fatty acids pattern observed in plasma from healthy males and females as a function of time and the consumption of ALA. No sex effect observed. Values represent the mean±SEM. A) ALA (alpha-linolenic acid). B) EPA (eicosapentaenoic acid). C) DHA (docosahexaenoic acid) D) Total plasma omega-3 fatty acids. (*) Significant difference between baseline and time point for the same sex (#) Significant difference between sexes for the same time point ($p < 0.05$).

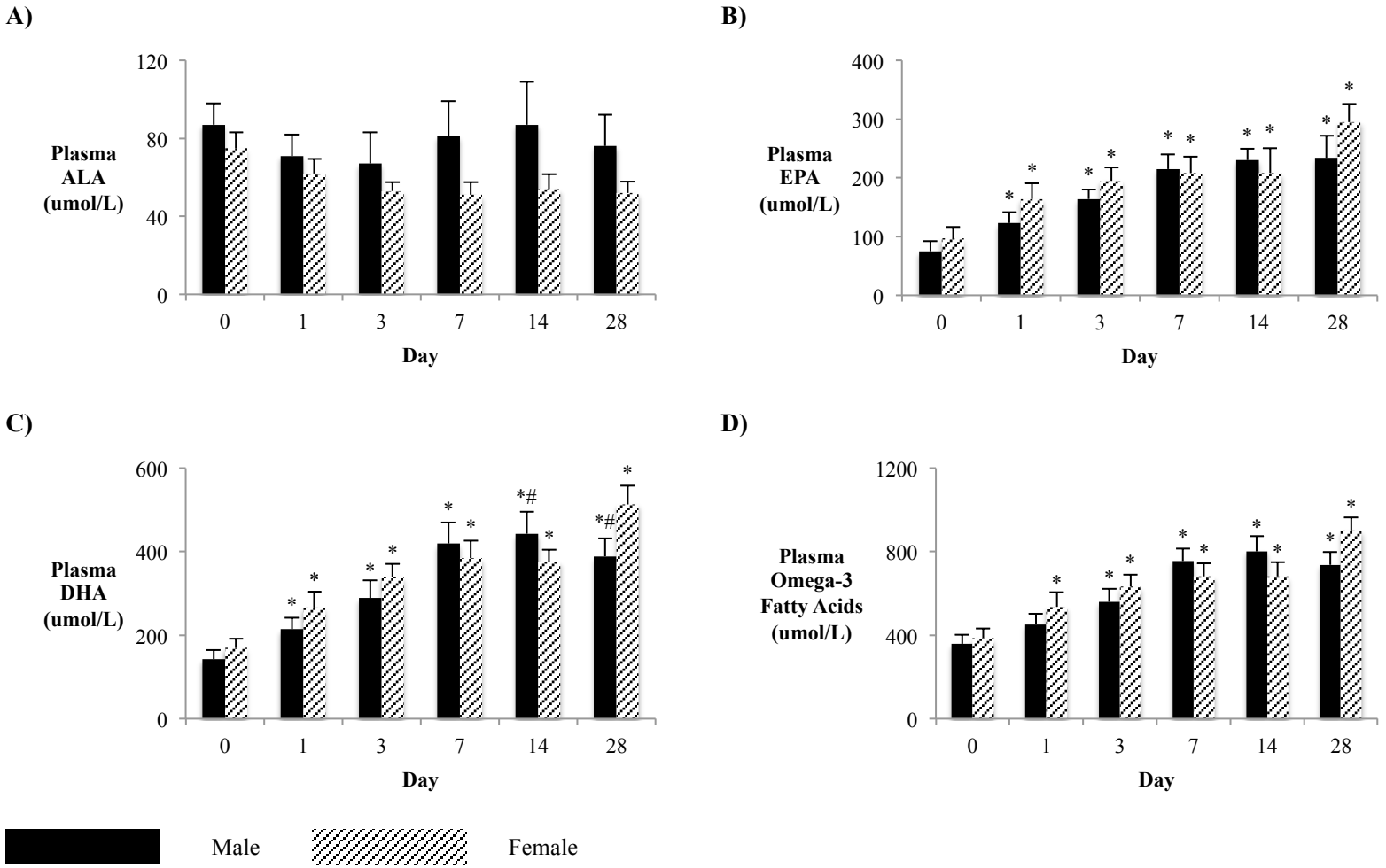


Figure 2-3. Select omega-3 plasma fatty acids among healthy individuals consuming DHA oil.
Select omega-3 fatty acids pattern observed in plasma from healthy males and females as a function of time and the consumption of flax oil. No sex effect observed. Values represent the mean±SEM. A) ALA (alpha-linolenic acid). B) EPA (eicosapentaenoic acid). C) DHA (docosahexaenoic acid) D) Total plasma omega-3 fatty acids. (*) Significant difference between baseline and time point for the same sex (#) Significant difference between sexes for the same time point ($p < 0.05$).

and 6 days for DHA whereas males reached a plateau at 8 days for both of these fatty acids (Appendix 4.7 **Table 0-14**). Differences were observed for total omega-3 fatty acids where females displayed higher than baseline total omega-3 fatty acids at day 1 onwards and males displayed higher than baseline total omega-3 fatty acids at day 3 onwards (**Figure 2-3**).

Analysis of males and females separately allow us to determine that the increases in total omega-6 fatty acids as well as total PUFA at days 7 and 28 we observe in response to ALA oil are exclusive to males (Appendix 4.7 **Table 0-6**). Further, males and females responded differently to DHA oil treatment with regards to DPAω6 with males having elevated levels from baseline from day 3 onwards and females only at days 1 and 3 (Appendix 4.7 **Table 0-7**). Results for all fatty acids are listed in Appendix 4.7 **Table 0-6** and **Table 0-7**.

Oxylipins

We screened for 167 unique oxylipins in serum, plasma, and both supplemental oils, and detected and quantified 77 different oxylipins. Oxylipins that originated from several PUFA precursors were quantified, including: LA (10), ALA (5), GLA (1), DGLA (4), AA (34), EPA (9), and DHA (14).

At baseline, LA derived oxylipins were present in the highest concentration (33257 pM for males, 33205 pM, for females). Total EPA (345 pM, for males, 357 pM, for females), and DHA (2075 pM, for males, 2866 pM for females) derived oxylipins were present in lesser concentrations compared to total LA derived oxylipins. Finally, total ALA derived oxylipins at baseline were present at 1171 pM in males and 916 pM in females at baseline.

Differences between treatments were observed in a few single oxylipins at baseline when the sexes were combined. Oxylipins present in higher concentrations at baseline in DHA oil

group include: 8-HETrE, LTE_4, 12,13-EpODE, and TXB_3 and oxylipins present in higher concentrations at baseline in ALA oil group include: PGF2α and 16-HETE (Appendix 4.7 Table 0-9). No groups of oxylipins (i.e. total DHA oxylipins, total LOX oxylipins) were significantly different between treatments when the sexes are combined (Appendix 4.7 Table 0-8). Further there were no differences between sexes for any single oxylipin (Table 2-3 Table 2-4) or group of oxylipins (Appendix 4.7 Table 0-10, Table 0-11) at baseline, however, several oxylipins and oxylipin groups had an overall sex effect and in all but one, females had higher concentrations than males over all time points. No major differences between treatments baseline were observed for ALA, EPA, and DHA derived oxylipins suggesting washout and wash in periods were of sufficient length.

Oxylipin alterations in response to ALA and DHA oil treatment

DHA supplementation at 4 g/day caused increases in total EPA, DHA, and omega-3 derived oxylipins from baseline at day 28, however, supplementation of 4 g/day of ALA had no effect on total ALA or total omega-3 derived oxylipins when sexes are combined (Appendix 4.7 Table 0-8). These results are further supported when examining individual oxylipins. In response to DHA oil treatment 5 of 7 EPA derived and all DHA derived oxylipins were increased from baseline at day 28 (Appendix 4.7 Table 0-9). Increased total LA oxylipins and total omega-6 oxylipins was observed at day 28 in response to DHA oil treatment. Further, in response to ALA oil treatment, only 1 of 5 ALA derived oxylipins (13-HOTrE) was increased from baseline at day 28 (Appendix 4.7 Table 0-9).

Increases in total EPA, DHA and omega-3 fatty acids from baseline were not due to increases in specific enzymatic production pathways (i.e. COX, LOX, cP450) as all pathways were elevated for EPA and DHA in response to DHA oil treatment when sexes are combined.

Further, DHA oil treatment increased total plasma oxylipins at day 28. No pathway alterations from baseline at day 28 were observed in response to ALA treatment. These results are further supported by individual oxylipins and all results are listed in Appendix 4.7 Table 0-8 and Table 0-9.

Oxylipin time course alterations in response to ALA and DHA oil treatments

Supplementation of DHA at 4 g/day caused increases in total EPA, DHA, and omega-3 derived oxylipins beginning at day 1 and throughout the treatment period, whereas ALA at 4 g/day did not significantly increase total ALA, EPA, DHA or total omega-3 fatty acids over this time course when sexes are combined (Appendix 4.7 Table 0-8, Figure 2-4). Further, alterations in specific enzymatic production pathways were not observed when examining increases in total EPA, DHA and omega-3 fatty acids from baseline and over the time course as all pathways were elevated in response to DHA oil treatment (Appendix 4.Table 0-8). Increases in total LA and total omega-6 oxylipins were not observed over the time course and were only elevated at day 28 of DHA oil treatment. No pathway alterations from baseline and over the time course were observed in response to ALA treatment (Appendix 4.7 Table 0-8).

Heat map representation also shows these trends on an individual oxylipin basis (**Figure 2-5**). ALA oil treatment shows very few differences among individual and grouped oxylipins, however, it appears there is a slight increase in ALA derived oxylipins, although not significant (Appendix 4.7 Table 0-8 and Table 0-9). The heat map representation further confirms the increases in EPA and DHA derived oxylipins among those consuming DHA oil treatment, with many increases many beginning at day one. This trend is very predominant in the EPA derived HEPEs and DHA derived HDoHEs (**Figure 2-5**).

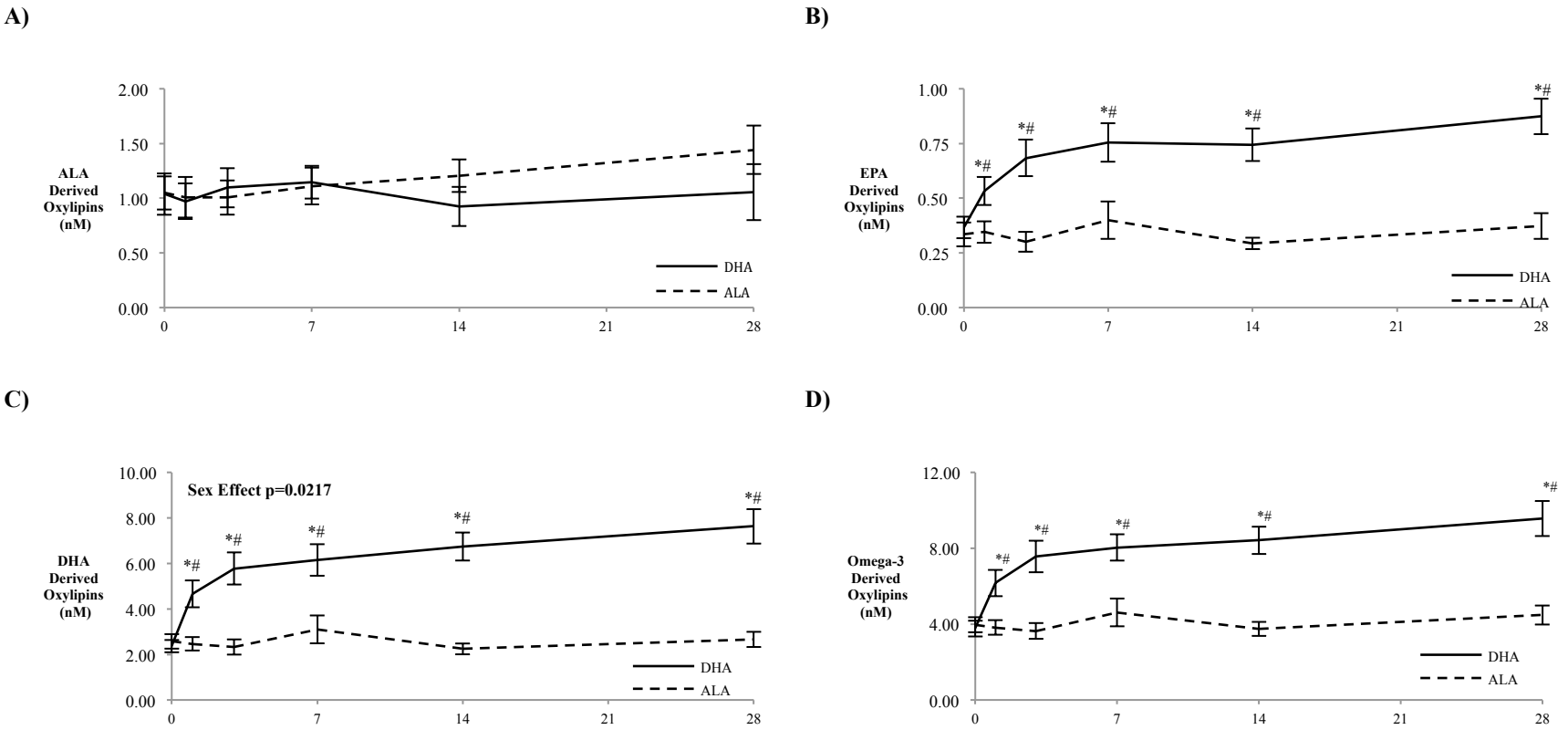


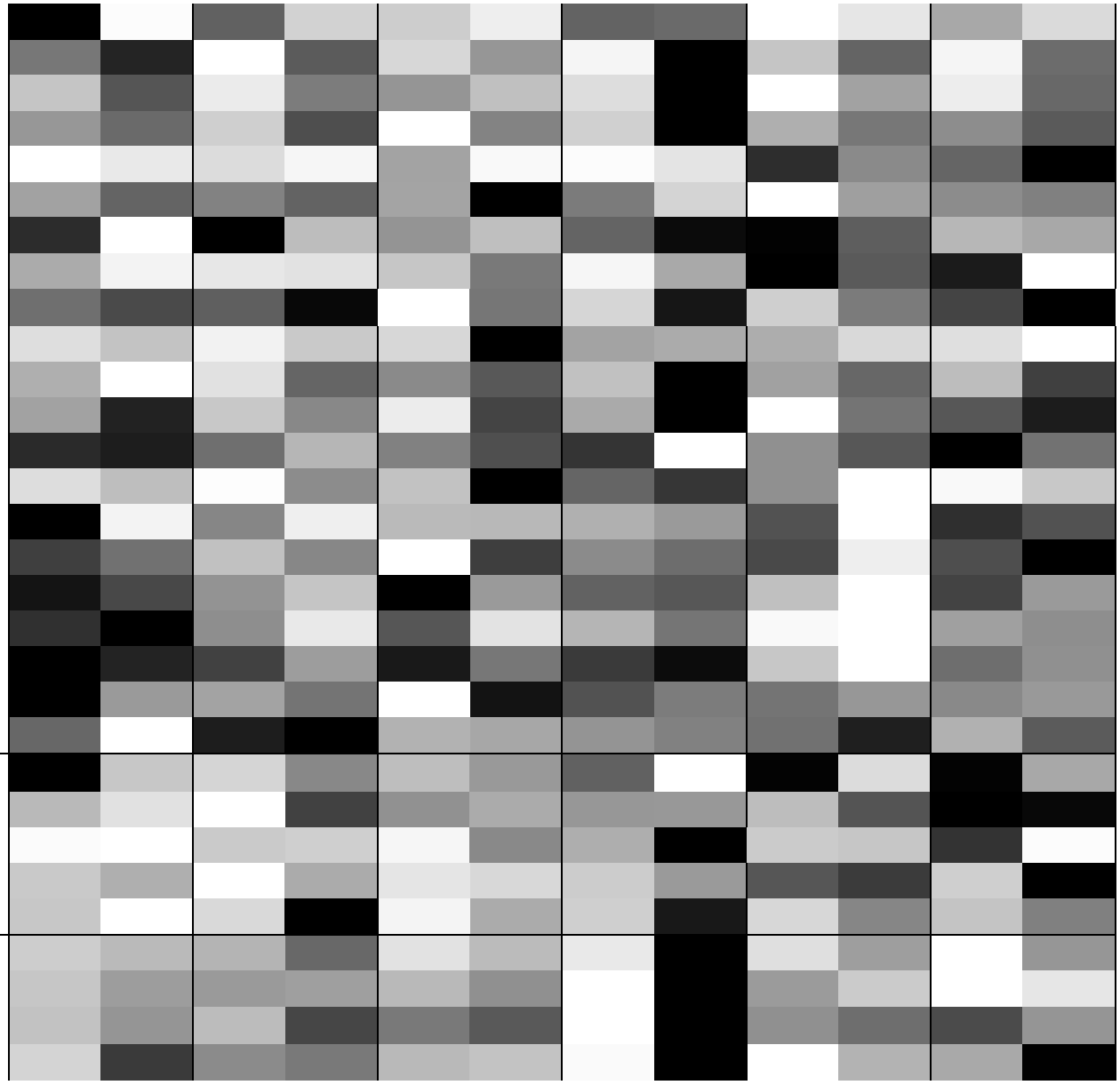
Figure 2-4. Select omega-3 derived oxylipins in plasma.

ALA, EPA DHA, and total omega-3 derived oxylipins in plasma from healthy participants as a function of time and treatment. Data represents sexes combined. Sex effect listed where significant. Values represent the mean±SEM. A) Total ALA derived oxylipins. B) Total EPA derived oxylipins. C) Total DHA derived oxylipins D) Total Omega-3 derived oxylipins.(*) Significant difference between baseline and time point for the same treatment. (#) Significant difference between treatments for the same time point ($p < 0.05$).

Figure 2-5. Heat maps for plasma oxylipins at all time points in ALA and DHA oil treatments.
Heat map presents visual representation of plasma analyte concentration where lighter shades indicate lower concentrations and darker shades indicates larger concentration within each analyte, across time points, and between sexes for ALA oil treatment (A) and DHA oil treatment (B).

A)

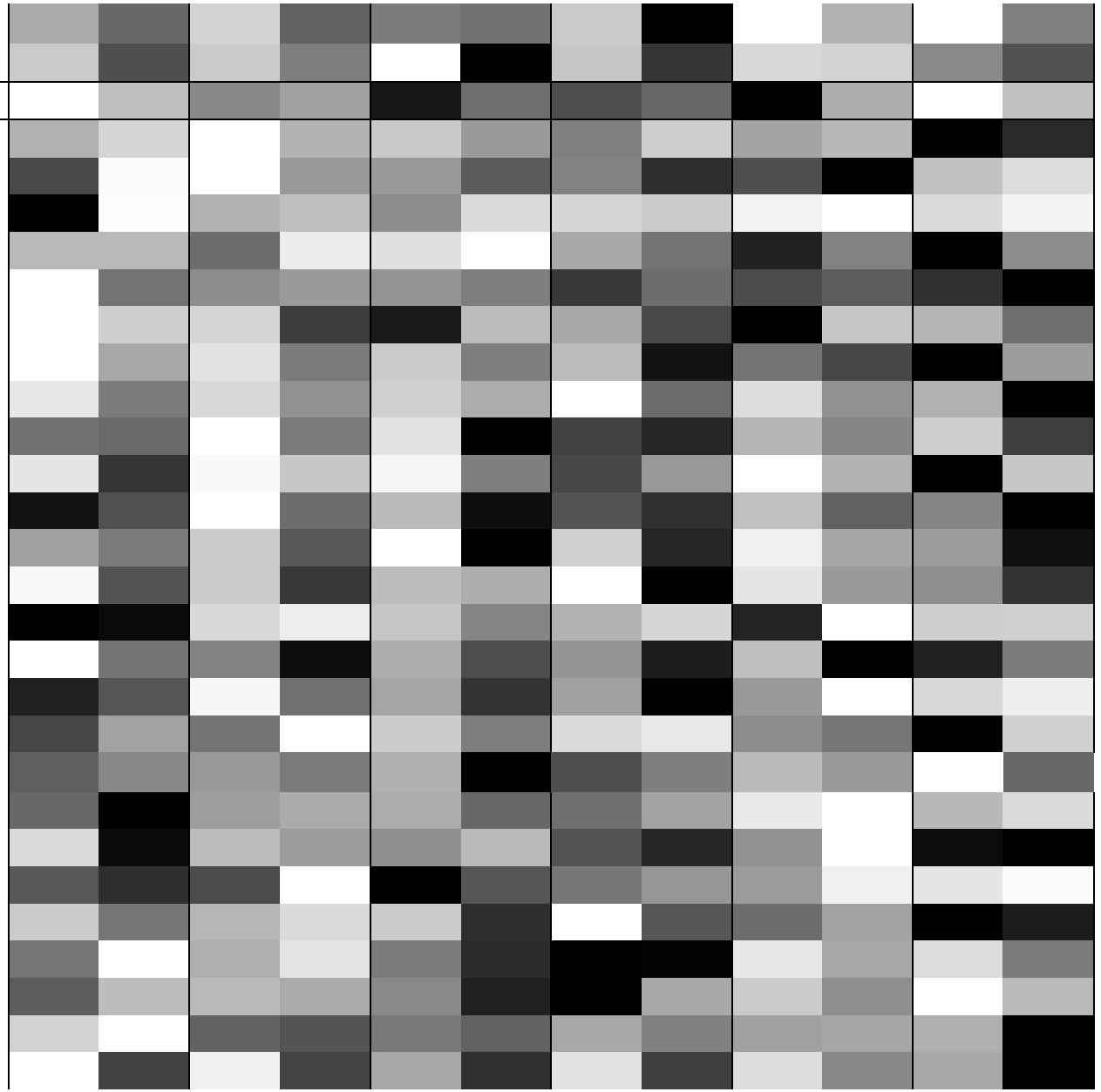
Fatty Acid	Enzyme	Oxylipin	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
LA	LOX	9-HODE												
LA	LOX	13-HODE												
LA	LOX	9-oxoODE												
LA	LOX	13-oxoODE												
LA	LOX	9,10,13 triHOME												
LA	LOX	9,12,13 triHOME												
LA	cP450	9,10 diHOME												
LA	cP450	9,10 EpOME												
LA	cP450	12,13 diHOME												
LA	cP450	12,13 EpOME												
GLA	LOX	13-HOTrE-γ												
DGLA	COX	$PGF_{1\alpha}$												
DGLA	LOX	8-HETrE												
DGLA	LOX	15-HETrE												
EDA	LOX	15-oxoEDE												
AA	COX	$11\beta PGE_2$												
AA	COX	12-HHTrE												
AA	COX	6k $PGF_{1\alpha}$												
AA	COX	PGD_2												
AA	COX	PGE_2												
AA	COX	$PGF_{2\alpha}$												
AA	COX	TXB_2												
AA	LOX	5-HETE												
AA	LOX	8-HETE												

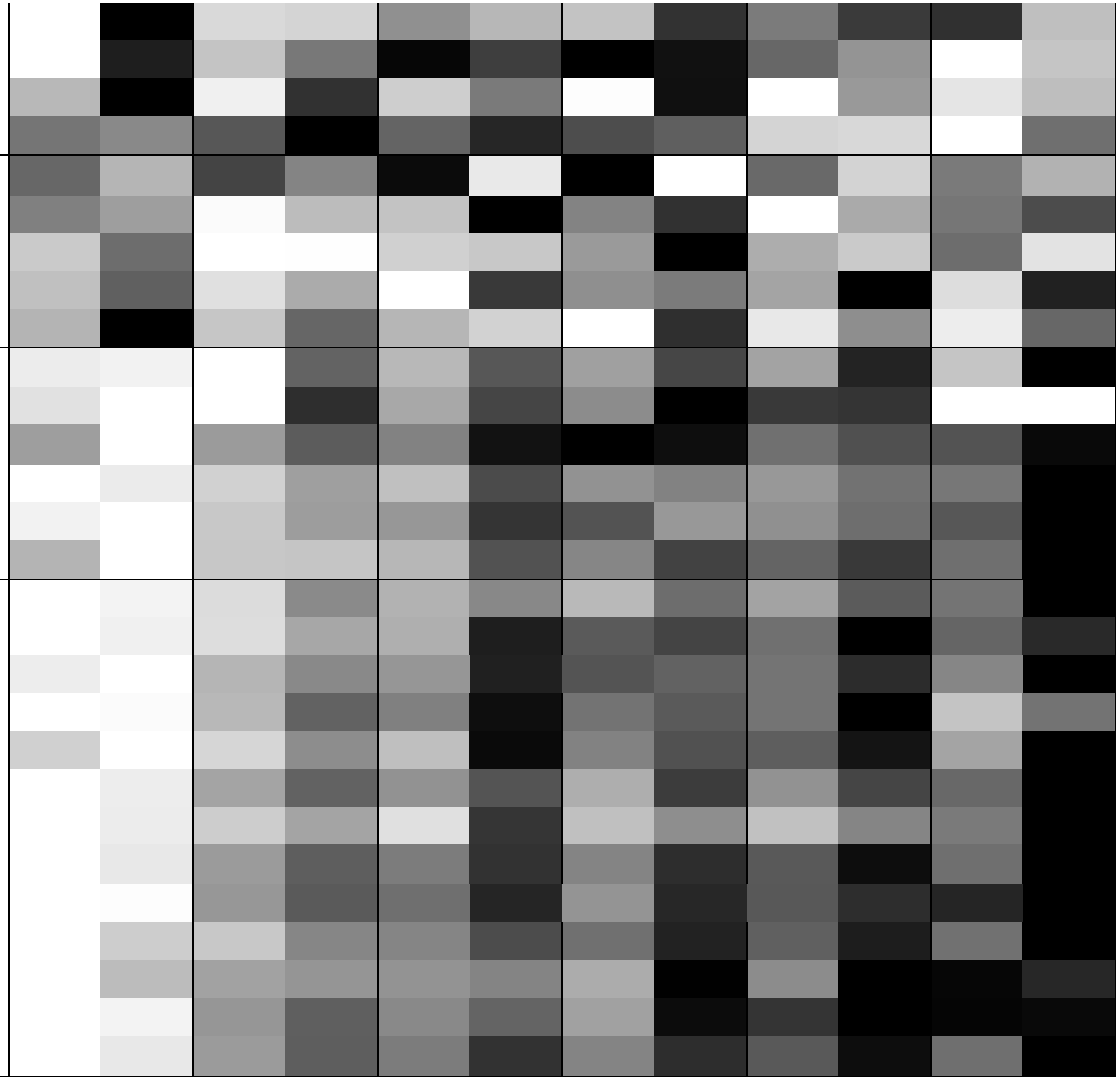
AA	LOX	9-HETE	
AA	COX/LOX	11-HETE	
AA	LOX	12-HETE	
AA	LOX	15-HETE	
AA	LOX	5-oxoETE	
AA	LOX	12-oxoETE	
AA	LOX	15-oxoETE	
AA	LOX	LTE_4	
AA	LOX	tetranor 12-HETE	
AA	cP450	5,6 DiHETrE	
AA	cP450	5,6 EpETrE	
AA	cP450	8,9 DiHETrE	
AA	cP450	11,12 DiHETrE	
AA	cP450	11,12 EpETrE	
AA	cP450	14,15 DiHETrE	
AA	cP450	14,15 EpETrE	
AA	cP450	16-HETE	
AA	cP450	17-HETE	
AA	cP450	18-HETE	
AA	cP450	20-HETE	
AA	non-enz	5-iso $PGF_{2\alpha}VI$	
ALA	LOX	9-HOTrE	
ALA	LOX	13-HOTrE	
ALA	LOX	9-oxoOTrE	
ALA	cP450	12,13 diHODE	
ALA	cP450	12,13 EpODE	
EPA	LOX	5-HEPE	
EPA	LOX	8-HEPE	
EPA	LOX	9-HEPE	
EPA	LOX	12-HEPE	

EPA	LOX	15-HEPE	
EPA	cP450	18-HEPE	
DHA	LOX	4-HDoHE	
DHA	LOX	7-HDoHE	
DHA	LOX	8-HDoHE	
DHA	LOX	10-HDoHE	
DHA	LOX	11-HDoHE	
DHA	COX/LOX	13-HDoHE	
DHA	LOX	14-HDoHE	
DHA	LOX	16-HDoHE	
DHA	LOX	17-HDoHE	
DHA	cP450	19,20 DiHDPE	
DHA	cP450	19,20 EpDPE	
DHA	cP450	20-HDoHE	

B)

			Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Fatty Acid	Enzyme	Oxylipin	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
LA	LOX	9-HODE												
LA	LOX	13-HODE												
LA	LOX	9-oxoODE												
LA	LOX	13-oxoODE												
LA	LOX	9,10,13 triHOME												
LA	LOX	9,12,13 triHOME												
LA	cP450	12,13 diHOME												
LA	cP450	12,13 EpOME												
LA	cP450	9,10 diHOME												
LA	cP450	9,10 EpOME												
GLA	LOX	13-HOTrE-γ												
DGLA	COX	$PGF_{1\alpha}$												

DGLA	LOX	8-HETrE	
DGLA	LOX	15-HETrE	
EDA	LOX	15-oxoEDE	
AA	COX	11βPGE$_2$	
AA	COX	12-HHTrE	
AA	COX	6k PGF$_{1\alpha}$	
AA	COX	PGD$_2$	
AA	COX	PGE$_2$	
AA	COX	PGF$_{2\alpha}$	
AA	COX	TXB$_2$	
AA	LOX	5-HETE	
AA	LOX	8-HETE	
AA	LOX	9-HETE	
AA	COX/LOX	11-HETE	
AA	LOX	12-HETE	
AA	LOX	15-HETE	
AA	LOX	5-oxoETE	
AA	LOX	12-oxoETE	
AA	LOX	15-oxoETE	
AA	LOX	LTE$_4$	
AA	LOX	tetranor 12-HETE	
AA	cP450	11,12 DiHETrE	
AA	cP450	11,12 EpETrE	
AA	cP450	14,15 DiHETrE	
AA	cP450	14,15 EpETrE	
AA	cP450	16-HETE	
AA	cP450	17-HETE	
AA	cP450	18-HETE	
AA	cP450	20-HETE	

AA	cP450	5,6 DiHETrE	
AA	cP450	5,6 EpETrE	
AA	cP450	8,9 DiHETrE	
AA	non-enz	5-iso $PGF_{2\alpha}VI$	
ALA	LOX	9-HOTrE	
ALA	LOX	13-HOTrE	
ALA	LOX	9 oxoOTrE	
ALA	cP450	12,13 diHODE	
ALA	cP450	12,13 EpODE	
EPA	LOX	5-HEPE	
EPA	LOX	8-HEPE	
EPA	LOX	9-HEPE	
EPA	LOX	12-HEPE	
EPA	LOX	15-HEPE	
EPA	cP450	18-HEPE	
DHA	LOX	4-HDoHE	
DHA	LOX	7-HDoHE	
DHA	LOX	8-HDoHE	
DHA	LOX	10-HDoHE	
DHA	LOX	11-HDoHE	
DHA	COX/LOX	13-HDoHE	
DHA	LOX	14-HDoHE	
DHA	LOX	16-HDoHE	
DHA	LOX	17-HDoHE	
DHA	cP450	19,20 DiHDPE	
DHA	cP450	19,20 EpDPE	
DHA	cP450	20-HDoHE	
DHA	LOX	16-HDoHE	

Sex specific alterations in oxylipin profiles in response to ALA and DHA oil treatments

In response to DHA oil treatment, males and females show alterations in several oxylipins from baseline at different times whereas response to ALA oil treatment is more varied in males and females. Total DHA oxylipins were all elevated at day one and throughout treatment for both sexes (Table 2-4). Sex differences in EPA derived oxylipins did not follow the same pattern: total EPA oxylipins were elevated beginning at day one throughout treatment in females where as males had elevated levels beginning at day seven. Further, total omega-3 derived oxylipins were elevated from baseline at day one and throughout treatment in females and at day three and throughout treatment for males. No obvious trends were observed when looking at ALA derived oxylipins, however, differences between sexes were observed at day one of ALA oil treatment with females having higher total ALA oxylipins compared to males (**Table 2-3**).

Heat map representation shows the trends we observed in individual oxylipins, specifically that we see several distinct alterations in response to DHA oil and very minimal alterations in response to ALA oil treatment (**Figure 2-5**, **Table 2-3** and Table 2-4). Heat map representation shows females consuming DHA oil consistently have higher levels of individual EPA and DHA derived oxylipins compared to males at the same time points (**Figure 2-5**, Table 2-4). While total DHA oxylipins are increased from baseline at day 1 for both males and females, alterations are observed when examining these oxylipins individually. Increases from baseline occur within the first week are often seen beginning at day one for females while males tend to show differences from baseline beginning at day 3 or 7 (**Figure 2-5**, **Table 2-3** and Table 2-4). These sex differences at specific time points for several individual oxylipins are observed in the DHA derived HDoHEs: females have increased levels of both DHA derived 4-HDoHE at day 28

Table 2-3. Plasma oxylipins for individuals consuming ALA oil treatment (pM).
(*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Oxylipin	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
LA - LOX Oxylipins												
9-HODE[2]	8026 ± 616	10084 ± 1558	6554 ± 618	10813 ± 1251	7128 ± 773	8438 ± 1022	8532 ± 1509	12987 ± 2685	8045 ± 1656	8241 ± 1177	8224 ± 1273	8928 ± 1611
13-HODE[2]	4243± 459	5466 ± 1042	3930 ± 423	5943 ± 804	3295 ± 205	4131 ± 393	4305 ± 598	6873 ± 1024	3897 ± 688	4690 ± 530	4101 ± 568	4720 ± 862
9-oxoODE	107 ± 30	83 ± 16	63 ± 27	122 ± 20	34 ± 16	94 ± 34	91 ± 29	169 ± 50	109 ± 30	127 ± 31	124 ± 51	104 ± 35
13-oxoODE[2]	578 ± 235	376 ± 145	524 ± 222	789 ± 213	453 ± 124	730 ± 153	818 ± 228	1032 ± 316*	494 ± 141	696± 128	754 ± 195	533 ± 135
9,10,13 triHOME[4]	5925 ± 1093	6149 ± 1390	7802 ± 1342	8124 ± 2605	11229 ± 3985	5571 ± 996	6422 ± 1573	17783 ± 10002	5858 ± 1432	6043 ± 1208	8790 ± 2552	4873 ± 1417
9,12,13 triHOME[4]	6520 ± 972	7656 ± 1441	6745 ± 1201	9164 ± 2801	12911 ± 4814	6861 ± 820	8448 ± 2522	12714 ± 4755	6589 ± 1718	8119 ± 1623	10772 ± 3145	5472 ± 1422
LA – cP450 Oxylipins												
9,10 diHOME[2]	237 ± 45	314 ± 41	230 ± 46	326 ± 59	220 ± 34	295 ± 60	255± 51	366 ± 109	195 ± 36	292 ± 61	195 ± 30	280 ± 74
9,10 EpOME[2]	41 ± 17	91 ± 18	31 ± 9.5	98 ± 23.67	36 ± 14	119 ± 41	51 ± 19	118 ± 32	78 ± 22	50 ± 20	80 ± 33	67 ± 22
12,13 diHOME[3]	416 ± 96	475 ± 109	387 ±112	566 ± 123	317 ± 43	435 ± 81	388 ± 79	559 ± 118	268 ±52*	421 ± 81	365 ± 124	389 ± 59
12,13 EpOME	173 ± 31	173 ± 40	127 ± 16	269 ± 78	115 ± 28	144 ± 32	150 ± 32	278 ± 74	209 ±125	136 ± 41	187 ± 95	272 ±103
Total LA Oxylipins	26266± 2509	30867± 4775	26392± 2454	36214± 7384	35738± 8377	26819± 2684	29460± 4561	52878± 15999	25742± 5349	28814± 4080	33592± 6519	25637± 4554
GLA – LOX Oxylipins												
13-HOTrE-γ	103 ± 20	108 ± 3.8	90 ± 20	98 ± 25	70 ± 14	90 ± 17	93 ± 19	111 ± 22	100 ± 19	97 ± 16	131 ± 29	125 ± 18
DGLA – COX Oxylipins												
$PGF_{1\alpha}$[4]	179 ± 53	173 ± 61	107 ± 35	121 ± 40	194 ± 101	123 ± 38	227 ± 74	121 ± 63	159 ± 52	50 ± 14*	275 ± 94	69 ± 10*
DGLA – LOX Oxylipins												
8-HETrE[2]	15 ± 5.9	1.2 ± 1.2	26 ± 6.2	38 ± 7.5*	13 ± 3.7	22 ± 5.5	15 ± 4.8†	54 ± 14*	8.7 ± 3.6*†	26 ± 7.6*	23 ± 7.7	33 ± 8.2*
15-HETrE[3]	130 ± 21	180 ± 12	106 ± 16	134 ± 14*	132 ± 14	142 ±20*	142 ± 16	192 ± 29	134± 20*†	132 ±22*	172 ± 29*†	157 ±20*

Total DGLA Oxylipins	428±108	355±58	377±150	293±29	339±100	286±33	384±88	366±85	415±121	208±25*	470±93	259±23
EDA – LOX Oxylipins												
15-oxoEDE	0.5 ± 0.3	--	0.9 ± 0.9	2.0 ± 1.2	4.9 ± 3.3	6.8 ±2.8*	2.4 ±1.7†	11 ± 10*	4.6 ± 4.1	2.5 ± 2.0	2.5 ± 2.5	1.5 ± 1.3
AA – COX Oxylipins												
11βPGE_2[3,4]	26 ± 4.6	22 ± 2.3	25 ± 1.5	31 ± 4.4*	21 ± 2.6	20 ± 2.3	22 ± 3.4	23 ± 2.7	25 ± 2.1	23 ± 2.6	29 ± 6.7	26 ± 2.9
12-HHTrE	180 ± 94	37 ± 29	19 ± 19*	21 ± 10	217 ± 179	56 ± 43	51 ± 33	76 ± 54	35 ± 20	85 ± 61	147 ± 123	44 ± 44
6k $PGF_{1\alpha}$[3]	13 ± 4.3	9.2 ± 3.5	11 ± 5.6	2.8 ± 1.0	8.2 ± 4.4	6.9 ± 2.3	4.4 ±1.2*	8.1 ± 3.8	5.9 ±1.4*	8.5 ± 3.2	13 ± 2.6	5.3 ± 1.3
PGD_2[4]	23 ± 5.1	12 ± 2.8	23 ± 4.2	10 ± 2.8	8.6 ± 4.7	4.8 ± 1.8	16 ± 7.5	11 ± 4.5	16 ± 3.8	9.0 ± 3.4	16 ± 6.8	16 ± 8.4
PGE_2[3]	19 ± 2.8	13 ± 0.7	18 ± 2.3	18 ± 1.7	14 ± 0.7	13 ± 0.9	14 ± 2.5	17 ± 4.8	16 ± 0.8	13 ± 2.1	20 ± 3.9	16 ± 0.8
$PGF_{2\alpha}$	53 ± 14	44 ± 6.7	31 ± 12	14 ± 5.3*	12 ± 2.1*	26 ± 1.0	22 ± 3.5*	19 ± 6.8	13 ± 2.5*	21 ± 2.4	46 ± 14	33 ± 6.4
TXB_2	19 ± 13	19 ± 9.8	6.7 ± 2.3	8.9 ± 3.5	18 ± 13	25 ± 14	14 ± 9.7	8.3 ± 3.9	15 ± 4.7	9.9 ± 3.6	9.2 ± 4.8	9.4 ± 4.6
AA - LOX Oxylipins												
5-HETE[2,3]	220 ± 27	345 ± 65	205 ± 45	355 ± 73	154 ± 7.2	268 ± 30	171 ± 15	388 ± 94	171 ± 27	280 ± 54	205 ± 31	287 ± 43
8-HETE[2,4]	303 ± 21	439 ± 36	271 ± 31	435 ± 47	302 ± 51	430 ± 51	319 ± 25	555 ± 121	337 ± 47	406 ± 49	304 ± 42	444 ± 51
9-HETE[2]	458 ±269	126 ± 14	331 ±181	182 ± 32	188 ± 73	144 ± 31	328 ±210	319 ± 125	122 ±11*	156 ± 40	237 ± 109	172 ± 68
11-HETE[1]	442 ± 84	536 ± 95	286 ± 81	474 ± 78	333 ± 56	407 ± 40	299 ± 50	577 ± 82	353 ± 48	463 ± 62	298 ± 40	454 ± 87
12-HETE[2,4]	362 ± 93	508 ± 44	312 ± 81	457 ± 71	424 ± 116	368 ± 28	330 ± 59	618 ± 95	286 ± 52	407 ± 55	309 ± 60	483 ± 96
15-HETE	333 ± 38	372 ± 34	285 ± 62	396 ± 59	244 ± 8.5	351 ± 44	285 ± 19	463 ± 82	313 ± 37	361 ± 44	343 ± 50	386 ± 55
5-oxoETE	76 ± 9.3	167 ± 54	218 ±105	112 ± 38	442 ± 311	103 ± 9.4	92 ± 12	184 ± 56	908 ±829	540± 458	688 ± 592	1084± 1025
12-oxoETE[2]	5.9 ± 3.3	8.0 ± 4.8	7.0 ± 4.0	8.0 ± 3.0	5.8 ± 2.8	11 ± 3.7	7.2 ± 2.6	4.2 ± 1.9	2.8 ± 1.4	6.0 ± 2.2	6.6 ± 3.7	7.0 ± 1.0
15-oxoETE	33 ± 8.7	20 ± 3.5	36 ± 7.1	24 ± 3.4	27 ± 9.8	24 ± 2.9	30 ± 11	35 ± 8.0	36 ± 9.3	30 ± 4.0	25 ± 3.0	26 ± 2.8
LTB_4	--	--	--	--	--	1.3 ± 1.3	0.9 ± 0.9	0.4 ± 0.4	--	--	3.6±3.6*†	--
LTE_4[4]	1.3 ± 1.3	0.3 ± 0.3	0.5 ± 0.5	0.5 ± 0.5	0.9 ± 0.9	2.0 ± 2.0	0.2 ± 0.2	1.3 ± 0.8	3.8 ± 2.0	2.5 ± 2.5	3.4 ± 3.4	0.1 ± 0.1
tetranor 12-HETE	88 ± 13	98 ± 23	92 ± 18	115 ± 29	51 ± 19	86 ± 21	61 ± 17	111 ± 28	63 ± 13	85 ± 14	99 ± 40	117 ± 29
AA – cP450 Oxylipins												
5,6 DiHETrE	67 ± 11	76 ± 6.9	61 ± 9.2	74 ± 13	69 ± 25	140 ± 41	86 ± 30	84 ± 11	83 ± 24	69 ± 14	67 ± 14	56 ± 6.1
5,6 EpETrE	1.4 ± 1.0	--	0.5 ± 0.5	2.7 ± 1.8	2.1 ± 1.6	2.9 ± 1.3	1.1 ± 1.1	4.5 ± 1.8*	1.7 ± 1.2	2.7 ± 1.8	1.2 ± 1.2	3.4 ± 1.8
8,9 DiHETrE[2]	30 ± 3.2	35 ± 2.2	28 ± 2.0	31 ± 3.4	27 ± 3.2	34 ± 2.8*	30 ± 2.8	36 ± 5.6	26 ± 2.3	32 ± 2.4	33 ± 3.3	35 ± 3.9
11,12 DiHETrE[3]	131 ± 16	132 ± 14	123 ± 10	114 ± 6.7	121 ± 15	126 ± 6.5	130 ± 11	106 ± 10	119 ± 14	125 ± 9.9	135 ± 16	122 ± 11

11,12 EpETrE	10 ± 1.5	11 ± 1.3	9.3 ± 2.5	13 ± 2.7	11 ± 3.0	17 ± 5.7	14 ± 5.2	15 ± 2.8	13 ± 3.5	9.3 ± 0.9	9.5 ± 2.3	11 ± 1.5
14,15 DiHETrE	204 ± 24	151 ± 15	175 ± 16	152 ± 12	163±14*†	164 ± 20	166± 10*†	170 ± 18	186 ± 23	148 ± 10	194 ± 16	186 ± 22
14,15 EpETrE	43 ± 13	38 ± 8.5	29 ± 6.8	35 ± 8.1	22 ± 5.1	44 ± 13	35 ± 9.2	38 ± 11	42 ± 12	24 ± 5.2	42 ± 16	50 ± 5.5
16-HETE[3]	204 ± 46	181 ± 27	148 ± 35	126 ± 28	213 ± 88	145 ± 17	170 ± 44	174 ± 64	128 ± 48	100 ± 22	183 ± 88	145 ± 30
17-HETE	67 ± 11	73 ± 14	55 ± 7.8	44 ± 3.9*	62 ± 17	45 ± 3.6*	51 ± 4.8	58 ± 9.2	42 ± 6.8*	41 ± 3.4*	53 ± 16	55 ± 10
18-HETE[4]	101 ± 10	97 ± 11	94 ± 8.5	84 ± 9.1	99 ± 18	88 ± 12	95 ± 4.4	100 ± 7.0	79 ± 4.9	73 ± 3.6	89 ± 12	85 ± 15
20-HETE[2]	587 ± 91	447 ± 49	438 ± 57	481 ± 92	354±45*†	571 ±114	513 ± 96	474 ± 30	481 ± 74	449 ± 51	463 ± 56	447 ± 47
AA – Non-enzymatic Oxylipins												
5-iso $PGF_{2\alpha}VI$	26 ± 9.1	11 ± 5.2	34 ± 3.0	36 ± 11*	19 ± 6.0	20 ± 11	22 ± 8.4	24 ± 5.6	25 ± 9.8	33 ± 8.7*	19 ± 4.2	28 ± 8.8
Total AA Oxylipins	4130± 447	4029±300	3372± 295	3857±458	3632±443	3745± 311	3376± 318	4709±515	3947± 855	4012± 640	4137±816	4833± 1386
ALA - LOX Oxylipins												
9-HOTrE	620± 144	255 ± 170	228± 104*†	370 ± 235	271 ± 229	339 ±165	441 ±184	150 ± 105	616 ±135	215 ±156	616 ± 267	311 ±203
13-HOTrE	507 ± 65	457 ± 111	419 ± 66	656 ± 138	557 ± 119	524 ±108	550 ±124	547 ± 105	502 ± 65	632 ±161	737 ± 127	727 ±67*
9 oxoOTrE[3]	43 ± 21	40 ± 7.0	76 ± 18	73 ± 25	47 ± 30	120 ± 51	95 ± 53	213 ± 83*	76 ± 38	79 ± 24	179 ± 70*	42 ± 14
ALA – cP450 Oxylipins												
12,13 diHODE	59 ± 20	71 ± 26	36 ± 10	72 ± 23	47 ± 8.0	53 ± 14	58 ± 13	80 ± 31	109 ± 69	120 ± 78	56 ± 27	146 ± 61
12,13 EpODE[2]	28 ± 4.0	18 ± 3.8	25 ± 5.5†	64 ± 11*	20 ± 1.3	33 ± 5.7	27 ± 8.1†	59 ± 7.5*	26 ± 5.5	40 ± 9.0*	29 ± 8.6	41 ± 8.9*
Total ALA Oxylipins	1257±176	840±232	783±103†	1235±348	942±253	1070±210	1171±337	1050±93	1328±223	1086±207	1617±352	1267±278
EPA - COX Oxylipins												
TXB_3	--	--	0.4 ± 0.4	--	0.8 ± 0.8	--	--	--	--	--	--	--
EPA - LOX Oxylipins												
5-HEPE[2]	57 ± 15	66 ± 19	69 ± 12	102 ± 35	48 ± 6.7	65 ± 19	45 ± 6.2†	148 ± 52*	50 ± 10	78 ± 14	35 ± 5.3	82 ± 22
8-HEPE[3,4]	2.1 ± 2.1	3.7 ± 2.3	3.8 ± 3.8	3.6 ± 2.4	2.6 ± 2.6	4.2 ± 4.2	--	9.5 ± 6.1	3.8 ± 3.2	2.0 ± 2.0	--	1.0 ± 1.0
9-HEPE[3,4]	42 ± 9.7	52 ± 11	44 ± 10	69 ± 16	58 ± 18	65 ± 18	29 ± 8.0	84 ± 22	53 ± 11	60 ± 11	68 ± 21	52 ± 12
12-HEPE[2,3,4]	53 ± 9.3	98 ± 26	74 ± 27	80 ± 15	61 ± 29	58 ± 19	42 ± 9.2	115 ± 50	40 ± 10	63 ± 9.7	65 ± 23	115 ± 34
15-HEPE[3,4]	55 ± 11	76 ± 24	53 ± 12	43 ± 11	53 ± 19	50 ± 14	54 ± 9.9	80 ± 23	56 ± 6.4	44 ± 7.4	86 ± 24	80 ± 23
EPA – cP450 Oxylipins												

18-HEPE[3,4]	69 ± 8.1	97 ± 31	69 ± 10	80 ± 11	59 ± 8.6	77 ± 14	70 ± 16	124 ± 24	63 ± 10	76 ± 7.2	66 ± 13	97 ± 14
Total EPA Oxylipins[3,4]	278±38	392±103	313±60	377±80	283±63	319±73	240±47	559±141	265±35	322±38	320±70	426±94
DHA - LOX Oxylipins												
4-HDoHE[2,3,4]	209 ± 37	411 ± 128	237 ± 95	338 ± 73	188 ± 30	260 ± 72	189 ± 58	504 ± 151	180 ± 35	275 ± 53	263 ± 66	274 ± 79
7-HDoHE[3,4]	159 ± 54	198 ± 17	149 ± 74	140 ± 22	103 ± 34	245 ± 77	117 ± 36	304 ± 100	189 ± 41	140 ± 42	286 ± 143	208 ± 64
8-HDoHE[3,4]	84 ± 8.9	138 ± 22	86 ± 17	145 ± 35	62 ± 10	155 ± 46	91 ± 14	251 ± 74	112 ± 13	125 ± 30	203 ± 105	134 ± 28
10-HDoHE[2,3,4]	40 ± 14	60 ± 10	29 ± 8.3	48 ± 11	32 ± 4.6	51 ± 14	32 ± 9.0	81 ± 31	37 ± 6.2	49 ± 13	53 ± 20	51 ± 13
11-HDoHE[2,3,4]	75 ± 25	96 ± 21	48 ± 11	94 ± 16	77 ± 22	92 ± 27	68 ± 20	164 ± 62	61 ± 12	107 ± 22	105 ± 47	110 ± 34
13-HDoHE[1,2,3,4]	43 ± 5.3	62 ± 18	33 ± 11	65 ± 15	28 ± 4.8	52 ± 15	30 ± 11	93 ± 24	27 ± 5.9	53 ± 11	38 ± 11	58 ± 14
14-HDoHE[2,3,4]	98 ± 19	284 ± 105	137 ± 60	191 ± 35	108 ± 29	137 ± 30	158 ± 68	240 ± 57	88 ± 25	160 ± 43	86 ± 18	172 ± 40
16-HDoHE[2,3,4]	84 ± 7.7	113 ± 24	86 ± 18	109 ± 23	70 ± 9.2	119 ± 35	82 ± 12	183 ± 50	79 ± 11	99 ± 20	87 ± 14	114 ± 26
17-HDoHE[3,4]	313 ± 58	339 ± 89	262 ± 55	341 ± 77	312 ± 97	282 ± 73	246 ± 32	553 ± 174	333 ± 50	246 ± 52	304 ± 40	389 ± 50
DHA – cP450 Oxylipins												
19,20 DiHDPE[2,3,4]	716 ± 56	1176 ± 163	779 ± 43	1107 ± 212	730 ± 52	1062± 205	793 ± 40	1393 ± 367	694 ± 81	1001 ± 152	854 ± 115	1091 ± 194
19,20 EpDPE[3,4]	16 ± 3.9	37 ± 12	20 ± 6.4	29 ± 9.1	9.6 ± 4.3	44 ± 19	22 ± 10	31 ± 9.1	32 ± 11	18 ± 2.9	30 ± 8.4	28 ± 6.4
20-HDoHE[3,4]	169 ± 6.5	238 ± 37	198 ± 25	261 ± 52	178 ± 41	250 ± 59	186 ± 27	406 ± 136	160 ± 18	229 ± 60	200 ± 33	186 ± 37
Total DHA Oxylipins[2,3,4]	2005± 174	3154±548	2064± 267	2867±517	1897±126	2749± 615	2013± 283	4203±1060	1991± 259	2503± 400	2510±456	2814± 521
Total Plasma Oxylipins[2,4]	34468± 3014	39745± 5304	33392± 2844	44943± 8250	42907± 7936	35085± 3134	36739± 4523	63888± 17310	33793± 5936	37045± 4813	42779± 7448	35362± 6226

[1]Produced by COX and LOX; [2]Sex Effect; [3]Time Effect; [4]Treatment Effect; (--) represents analyte was below limit of quantification
HETrE, hydroxyeicosatrienoic acid; oxoEDE, oxo-eicosadienoic acid; oxoOTrE, oxo-octadecatrienoic acid; triHOME, trihydroxy-octadecenoic acid.

Table 2-4. Plasma oxylipins for individuals consuming DHA oil treatment (pM).
(✱) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Oxylipin	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
LA - LOX Oxylipins												
9-HODE[2]	5992 ± 969	11399 ± 2228	6683 ± 1381	9916 ± 2439	7429 ± 932	10768 ± 2554	5478 ± 323	12280 ± 1835	5445 ± 783	9116 ± 1127	9397 ± 2093	15421 ± 3923*
13-HODE[2]	3613 ± 537	6309 ± 1018	3487 ± 509	5273 ± 1164	3736 ± 389	5973 ± 1243	3234 ± 283	6393 ± 774	2760 ± 358	4444 ± 380*	4132 ± 685	5748 ± 1112
9-oxoODE	41 ± 17	148 ± 33	153 ± 82	163 ± 56	109 ± 45	150 ± 19	59 ± 26	173 ± 57	109 ± 53	73 ± 21	173 ± 60*	161 ± 65
13-oxoODE[2]	496 ± 223	757 ± 305	651 ± 233	732 ± 265	422 ± 136	580 ± 228	99 ± 23	932 ± 331	302 ± 104	752 ± 152	340 ± 131	602 ± 267
9,10,13 triHOME[4]	8490 ± 2735	8619 ± 1137	5316 ± 1150	19756 ± 11582	4812 ± 1798	9124 ± 1945	8644 ± 2919	18659 ± 7326.	7985 ± 3145	10324 ± 5820	28674 ± 15112*	19588 ± 9942
9,12,13 triHOME[4]	20970 ± 12526	9104 ± 1817	5333 ± 904*†	19851 ± 11403	4960 ± 1441*	10654 ± 2497	9830 ± 3506	19347 ± 7876	9621 ± 3845	10580 ± 5466	30965 ± 13979	22215 ± 12580
LA – cP450 Oxylipins												
9,10 diHOME[2]	231 ± 45	367 ± 89	189 ± 17	356 ± 112	219 ± 39	322 ± 75	186 ± 42	410 ± 66	140 ± 19	291 ± 40	196 ± 31	427 ± 109
9,10 EpOME[2]	25 ± 6.2	85 ± 36	46 ± 16	54 ± 13	52 ± 10	89 ± 56	16 ± 2.9	107 ± 24	35 ± 15	75 ± 43	82 ± 36	73 ± 20
12,13 diHOME[3]	307 ± 98	567 ± 125	292 ± 63	420 ± 122	307 ± 90	518 ± 122	403 ± 103	523 ± 62	189 ± 26	404 ± 51*	239 ± 31	555 ± 155
12,13 EpOME	84 ± 6.8	186 ± 25	127 ± 24	217 ± 67	234 ± 78	322 ± 128	196 ± 77	251 ± 49	93 ± 29	316 ± 127	105 ± 22	310 ± 101
Total LA Oxylipins	40247± 14125	37542± 4494	22278± 2591	56738± 21471	22280± 4270	38500± 5461	28144± 6388	59075± 15324	26679± 6548	36374± 11377	74303± 28276*	62387± 23822
GLA - LOX Oxylipins												
13-HOTrE-γ	108 ± 21	170 ± 33	84 ± 20	132 ± 43	88 ± 14	116 ± 28	103 ± 17	124 ± 31	62 ± 5.7	107 ± 13*	122 ± 28†	101 ± 33*
DGLA - COX Oxylipins												
$PGF_{1\alpha}$[4]	166 ± 47	190 ± 66	295 ± 118*†	110 ± 36	241 ± 103†	151 ± 41	290 ± 105*†	142 ± 53	154 ± 59	193 ± 38	358 ± 105*†	167 ± 48
DGLA - LOX Oxylipins												
8-HETrE[2]	21 ± 6.9	28 ± 12	16 ± 4.0	29 ± 12	26 ± 7.3	27 ± 7.3	17 ± 2.1	40 ± 14	11 ± 7.6	20 ± 6.6	11 ± 11	26 ± 15
15-HETrE[3]	141 ± 17	178 ± 33	141 ± 22	164 ± 36	126 ± 17	201 ± 22	142 ± 11	185 ± 22	138 ± 10	139 ± 22	161 ± 32	177 ± 35

Total DGLA Oxylipins	328±45	395±73	453± 100*†	303±70	393±114	379±30	450±104	366±44	437±147	352±48	530±119*†	369±63
EDA - LOX Oxylipins												
15-oxoEDE	--	0.8 ± 0.8	1.6 ± 1.6	1.2 ± 0.8	3.0 ± 1.5	1.9 ± 1.4	2.3 ± 2.1	2.0 ± 1.3	3.3 ± 2.3	1.1 ± 0.8	--	0.8 ± 0.8
AA - COX Oxylipins												
11βPGE$_2$[3,4]	26 ± 6.1	23 ± 2.8	20 ± 3.1	26 ± 4.1	24 ± 4.0	28 ± 2.0	30 ± 2.9	24 ± 1.7	27 ± 2.5	26 ± 3.0	41 ± 4.1*†	38 ± 5.1*
12-HHTrE	94 ± 82	2.2 ± 2.2	--	53 ± 31	53 ± 24	85 ± 31	64 ± 57	108 ± 57	91 ± 60	131 ± 89	32 ± 32	18 ± 18
6k PGF$_{1\alpha}$[3]	23 ± 4.9	5.7 ± 3.3	11±5.1*†	10 ± 2.2	14±1.9*†	8.2 ± 1.3	8.5 ± 2.9*†	9.2 ± 4.2	6.5± 3.5*†	5.6 ± 1.5	8.2 ± 6.7*†	6.4 ± 2.0
PGD$_2$[4]	19 ± 12	19 ± 3.8	27 ± 12	13 ± 6.7	15 ± 6.8	11 ± 2.4	21 ± 6.9	27 ± 6.8	35 ± 16	25 ± 7.8	39 ± 13*	24 ± 5.8
PGE$_2$[3]	11 ± 3.1	18 ± 2.6	16 ± 1.3	16 ± 1.6	16 ± 1.8	17 ± 1.8	20 ± 2.4*†	18 ± 1.8	19 ± 1.6*	19 ± 1.8	21 ± 6.1*	23 ± 1.6
PGF$_{2\alpha}$	13 ± 5.6	21 ± 4.2	20 ± 5.8	43 ± 11	49 ± 17*	24 ± 5.3	27 ± 4.5	41 ± 21	53 ±24*†	22 ± 7.1	25 ± 4.8	36 ± 15
TXB$_2$	3.6 ± 2.4	14 ±6.7	7.1 ± 3.1	19.3 ± 9.0	9.8 ± 5.9	19 ± 7.2	12 ± 3.9	32 ± 10	20 ± 15	25 ± 9.8	34 ± 16*	15 ± 9.7
AA - LOX Oxylipins												
5-HETE[2,3]	165 ± 9.3	288 ± 52	182 ± 21	264 ± 43	192 ± 22	233 ± 20	137 ± 5.8	308 ± 14	176 ± 28	264 ± 20	227 ± 42	430 ± 95*
8-HETE[2,4]	458 ± 134	470 ±110	278 ±24*	448 ± 73	315 ±20†	603 ±125	521 ±123	556 ± 94	374 ± 68	432 ± 41	342 ± 76	524 ± 118
9-HETE	140 ± 38	386 ±196	112 ± 7.1	183 ± 25	116 ± 18	283 ± 42	360 ±154	248 ± 73	103 ± 13	211 ± 36	459±311*†	182 ± 52
11-HETE[1]	555 ± 180	482 ±124	272 ±26*	448 ± 95	355 ± 56	560 ±152	477 ± 90	521 ± 103	348 ± 58	460 ± 72	419 ± 154	577 ± 153
12-HETE[2,4]	449 ± 78	515 ± 51	378 ± 78	573 ± 79	288 ±32†	722± 148*	371 ± 45	659 ± 137	315 ± 36	441 ± 42	458 ± 100	697 ± 143
15-HETE	303 ± 20	406 ± 78	330 ± 18	422 ± 81	340 ± 37	349 ± 20	299 ± 19	456 ± 69	315 ± 28	361 ± 28	369 ± 29	425 ± 74
5-oxoETE	590 ± 496	569 ±454	142 ± 53	99 ± 26	182 ± 88	314 ±151	222 ±106	147 ± 37	518 ±356	60 ± 18	162 ± 61	158 ± 71
12-oxoETE[2]	--	9.0 ± 4.3	8.0 ± 3.1	15 ± 5.3	5.3 ± 2.6	11 ± 3.0	6.9 ± 3.2	15 ± 4.7	4.1 ± 2.4	16 ± 5.0	14 ± 8.6*†	8.4 ± 3.3
15-oxoETE	34± 7.2	31 ± 6.6	21 ± 3.9	29 ± 4.3	26 ± 4.0	33 ± 6.2	26 ± 6.1	36 ± 6.6	27 ± 7.4	21 ± 1.9	23 ± 7.12	22 ± 7.3
LTB$_4$	--	--	--	--	--	--	--	--	0.4 ± 0.4	--	--	--
LTE$_4$[4]	5.3 ± 3.1	2.7 ± 2.2	4.0 ± 1.9	--	1.5 ± 1.4	3.7 ± 3.4	1.1 ± 0.5	0.7 ± 0.4	3.3 ± 3.3	3.9 ± 3.7	7.3 ± 4.1	1.3 ± 1.0
tetranor 12-HETE	113 ± 27	94 ± 16	86 ± 18	100 ± 19	74 ± 13	158 ± 28	121 ± 26	98 ± 17	70 ± 16	85 ± 16	36 ± 9.2*	109 ± 36
AA – cP450 Oxylipins												
5,6 DiHETrE	47 ± 7.5	88 ± 15	54 ± 8.3	54 ± 7.1	65 ± 10	59 ± 14	57 ± 15	80 ± 26	69 ± 21	79 ± 39	81 ± 7.9	58 ± 11
5,6 EpETrE	--	3.3 ± 2.1	0.9 ± 0.9	2.0 ± 2.0	3.6 ± 2.6	2.8 ± 1.7	3.7 ± 3.1	3.5 ± 2.2	2.2 ± 1.8	1.6 ± 1.1	--	0.9 ± 0.9
8,9 DiHETrE[2]	30 ± 2.1	38 ± 4.1	27 ± 2.3	36 ± 3.9	29 ± 2.0	32 ± 1.9	26 ± 1.6	37 ± 3.0	26 ± 2.8	31 ± 3.8*	27 ± 2	29 ± 6.1*

11,12 DiHETrE[3]	133 ± 15	157 ± 18	120 ± 6.6	117 ± 7.3*	117 ± 6.8	133 ± 17	131 ± 11	119 ±8.3*	103± 10*†	97 ± 6.8*	114 ± 14	106 ± 7.4*
11,12 EpETrE	7.6 ± 0.7	16 ± 4.9	8.9 ± 1.0	10 ± 1.8	11 ± 1.1	8.9 ± 2.9	13 ± 3.6	15 ± 3.2	11 ± 2.7	6.2 ± 1.0*	16 ± 3.4*	16 ± 7.6
14,15 DiHETrE	182 ± 15	191 ± 20	185 ± 20	147 ± 8.1*	200 ± 16	183 ± 19	176 ± 11	169 ± 11	168 ± 20	151 ± 10*	153 ± 12	149 ± 5.5*
14,15 EpETrE	30 ± 3.6	41 ± 7.1	33 ± 6.5	28 ± 5.9	30 ± 4.7	50 ± 17	23 ± 5.9	45 ± 5.6	42 ± 19	35 ± 6.6	56 ± 19	53 ± 16
16-HETE[3]	157 ± 31	92 ± 21	130 ± 38	105 ± 8.6	155 ± 29	192 ± 39	213 ± 72	212 ± 41*	103 ± 34	134 ± 20	108 ± 15	154 ± 58
17-HETE	63 ± 12	49 ± 8.0	49 ± 7.2	51 ± 4.4	56 ± 8.4	72 ± 9.3	76 ± 22	51 ± 6.7	47 ± 5.3	55 ± 8.7	39 ± 7.8	49 ± 6.5
18-HETE[4]	95 ± 2.0	86 ± 5.4	115 ± 13	118 ± 13*	111 ± 13	116± 13*	103 ± 15	119 ± 9.4	104 ± 10	103 ± 6.3	101 ± 18†	134 ± 18*
20-HETE[2]	388 ± 39	563 ± 46	402 ± 62	562 ± 72	470 ± 23	580± 102	417 ± 47	564 ± 49	420 ± 40	498 ± 49	468 ± 110	622 ± 61
AA – Non-Enzymatic Oxylipins												
5-iso $PGF_{2\alpha}VI$	27 ± 10	25 ± 13	30 ± 8.8	40 ± 9.1	29 ± 8.2	36 ± 5.4	31 ± 8.0	29 ± 7.4	16 ± 4.3	16 ± 4.9	12 ± 7.4	28 ± 10
Total AA Oxylipins	4169± 1010	4703± 722	3070± 114	4038±415	3353± 237	4930± 471	3995± 383	4739±514	3618± 512	3816±187	3891±577	4696±524
ALA - LOX Oxylipins												
9-HOTrE	444 ± 190	259 ±208	527 ± 95	375 ± 230	664 ±188	133 ±133	689 ±133	81 ± 81	440 ±173	188 ± 129	400 ± 262	266 ± 266
13-HOTrE	509 ± 130	478 ± 53	380 ± 72	446 ± 109	439 ±100	644 ±204	506 ± 81	593 ± 145	375 ± 87	465 ± 67	520 ± 138	564 ± 125
9 oxoOTrE[3]	52 ± 10	99 ± 42	25 ± 11	26 ± 10	49 ± 16	52 ± 12	76 ± 27	153 ± 93	67 ± 37	52 ± 28	99 ± 51	39 ± 14
ALA – cP450 Oxylipins												
12,13 diHODE	54 ± 16	104 ± 39	38 ± 10	65 ± 16	21 ± 8.9	123 ± 64	79 ± 29	90 ± 15	69 ± 51	153 ± 85	39 ± 3.6	136 ± 51
12,13 EpODE[2]	26 ± 4.4	53 ± 14	23 ± 4.1	38 ± 8.8	25 ± 5.2†	21 ± 3.9*	14 ± 4.9	46 ± 13	17 ± 3.7	31 ± 7.0*	17 ± 5.1	37 ± 12
Total ALA Oxylipins	1085±292	992±266	992±142	950±312	1198± 296	974±198	1364± 176	963±215	968±309	889±230	1075±394	1043±372
EPA - COX Oxylipins												
TXB_3	1.5 ± 1.5	1.9 ± 1.9	--	--	--	--	--	--	--	--	1.9 ± 1.2	--
EPA – LOX Oxylipins												
5-HEPE[2,3,4]	83 ± 36	80 ± 23	74 ± 18†	152 ± 31*	109 ± 22	158± 21*	121 ± 33	166 ± 41*	120 ± 21	184 ± 31*	103 ± 20†	201 ± 64*
8-HEPE[3,4]	2.0 ± 2.0	--	--	14 ± 8.5*	5.8 ± 3.6	12 ± 7.6*	7.6 ± 4.7	17 ± 8.2*	13 ± 5.7	13 ± 8.9	--	--
9-HEPE[3,4]	83 ± 33	41 ± 9.2	84 ± 16	111 ± 10*	95 ± 26†	143± 23*	150 ± 69	144 ± 36*	103 ± 20	117 ± 26*	115 ± 48	147 ± 54*
12-HEPE[2,3,4]	50 ± 10	66 ± 11	87 ± 22	127 ± 26*	100 ± 25	192± 31*	137± 45*	149 ± 12*	132± 25*	162 ± 21*	157 ± 32*	251 ± 25*
15-HEPE[3,4]	60 ± 13	54 ± 9.2	80 ± 20	101 ± 21*	103± 19*	151± 30*	136± 20*	103 ± 15*	107± 15*	123 ± 23*	134 ± 30*	175 ± 30*
EPA – cP450 Oxylipins												

18-HEPE[3,4]	131 ± 35	78 ± 14	117 ± 16	119 ± 25	129 ±25†	199 ±36*	163 ±60†	210 ± 38*	187 ± 35	216 ± 36*	178 ± 19†	256 ± 52*
Total EPA Oxylipins[3,4]	411±83	322±55	442±78†	623±92*	543± 106†	854±89*	714± 168*	789±95*	661±92*	815±112*	689±117*†	1029±67*
DHA – LOX Oxylipins												
4-HDoHE[2,3,4]	184 ± 17	252 ± 68	376 ± 92	825 ±192*	606± 132*	836 ± 240*	567 ± 162*	982 ± 160*	687 ± 103*	1076 ± 166*	943 ± 305*†	1571 ± 329*
7-HDoHE[3,4]	212 ± 83	233 ± 42	260 ± 54	336 ± 61	325 ± 77	529± 94*	445± 30*	476 ± 85*	413± 109*	570±104*	429 ± 138*	513 ± 96*
8-HDoHE[3,4]	154 ± 79	118 ± 27	260 ± 79	343 ± 54*	318 ±70†	543 ±92*	444 ± 109*†	417 ± 64*	383 ±81*	521 ± 80*	349 ± 124*†	603 ± 125*
10-HDoHE[2,3,4]	39 ± 14	42 ± 11	93 ± 30	158 ± 39*	136 ±40*	223 ±64*	145 ±28*	164 ± 27*	145 ±28*	233 ± 23*	84 ± 7.0	146 ± 37*
11-HDoHE[2,3,4]	133 ± 64	78 ± 19	127± 37†	212 ± 48*	153 ±29†	365 ±67*	225 ± 40	282 ± 55*	266 ± 68*†	354 ± 23*	185 ± 5.6†	376 ± 77*
13-HDoHE[1,2,3,4]	31 ± 3.1	48 ± 7.6	113± 39*	173 ± 48*	129 ±40*	185 ±40*	104± 25*†	207 ± 44*	129 ±23*	198 ± 31*	167 ± 45*	260 ± 68*
14-HDoHE[2,3,4]	84 ± 11	139 ± 32	228 ± 60	343 ±112*	173 ±42†	661± 193*	266 ± 31	406 ± 91*	262 ± 32	432 ± 42*	463 ± 147*†	810 ± 174
16-HDoHE[2,3,4]	70 ± 5.1	100 ± 18	199 ±53*	277 ± 63*	238 ±73*	332 ±55*	229 ±46*	340 ± 44*	283 ±67*	380 ± 50*	255 ± 50*	396 ± 107*
17-HDoHE[3,4]	300 ± 38	307 ± 51	638 ± 124*	836 ± 167*	767 ± 206*	1006 ± 245*	647 ±72*	1001 ± 189*	841 ± 204*	981 ± 115*	1007±186*	1124 ± 309*
DHA – cP450 Oxylipins												
19,20 DiHDPE[2,3,4]	765 ± 110	1040 ± 139	1068 ± 127	1427 ± 145*	1430 ± 178*	1741 ± 175*	1546 ± 238*	1972 ± 274*	1632 ± 286*	1998 ± 140*	1540 ± 201*	2153 ± 199*
19,20 EpDPE[3,4]	15 ± 3.4	30 ± 6.5	35 ± 7.2	38 ± 6.1	39 ± 5.8	42 ± 14	33 ± 3.5	71 ± 18*	40 ± 18	71 ± 21*	70 ± 22*	62 ± 14*
20-HDoHE[3,4]	158 ± 15	189 ± 51	415 ± 75*	549 ± 128*	447 ± 89*	539 ± 68*	390 ± 125†	754 ± 177*	655 ± 175*	782 ± 135*	771 ± 243*	762 ± 261*
Total DHA Oxylipins[2,3,4]	2144±368	2577± 399	3811± 632*	5517± 936*	4761± 838*	7003± 997*	5036± 788*	7071± 983*	5736± 1020*	7595± 635*	6262± 1107*	8776±847*
Total Plasma Oxylipins[2,4]	48491± 14488	46701± 5382	31132± 3170	68301± 22320	32619± 5060	52757± 6912	39809± 6827	73129± 15871	38163± 6054	49950± 11091	86872± 28129*	78401± 23957*

[1]Produced by COX and LOX; [2]Sex Effect; [3]Time Effect; [4]Treatment Effect; (--) represents analyte was below limit of quantitation

and 20-HDoHE at day 7, when compared to males at the same time points (Figure 2-6).

Alterations observed in total grouped oxylipins in response to ALA oil treatment do not appear to follow a specific pattern however females displayed higher concentrations of several individual oxylipins. Females had higher 9-HOTrE than males at day one and higher 12,13-EpODE than males at day 1 and 7 (**Table 2-3**). Additionally, ALA derived 13-HOTrE was found in higher quantities than baseline for females at day 28, ALA derived 9-HOTrE decreased at day 1 for males, and ALA derived 12,13-EpODE was increased from baseline in females at days 1, 7, 14, and 28. Further, two ALA derived oxylipins,12,13-EpODE and 9-oxoOTrE, reached a plateau at day 5 in females (Appendix 4.7 **Table 0-15**). EPA derived 5-HEPE was elevated from baseline for females at day 7 and significantly higher than males also at day 7. However, there were no further changes seen for EPA or DHA derived oxylipins. Heat map representation does reveal a trend towards females consuming flax oil having higher DHA derived oxylipins than males, although this is not significant when looking at individual oxylipins (**Figure 2-5**, **Table 2-3**).

Calculating the time at which males and females reached plateau for individual and grouped oxylipins also revealed differences between sexes and these results were seen in response to DHA oil. Females reached a plateau for more analytes than males (4 of 7 EPA derived and 9 of 12 DHA derived products compared to 1 of 7 EPA derived and 3 of 12 DHA derived products in males) and also before males in several cases. Individual EPA derived oxylipins reached a plateau between 5-6 days for both males and females and individual DHA derived oxylipins reached a plateau between 5-7 days for females and 6-7 days for males. We were able to calculate a plateau for both males and females for three of these oxylipins (EPA derived 15-HEPE and DHA derived 8-HDoHE and 19,20-DiHDPE) and 2/3 reached a plateau in females

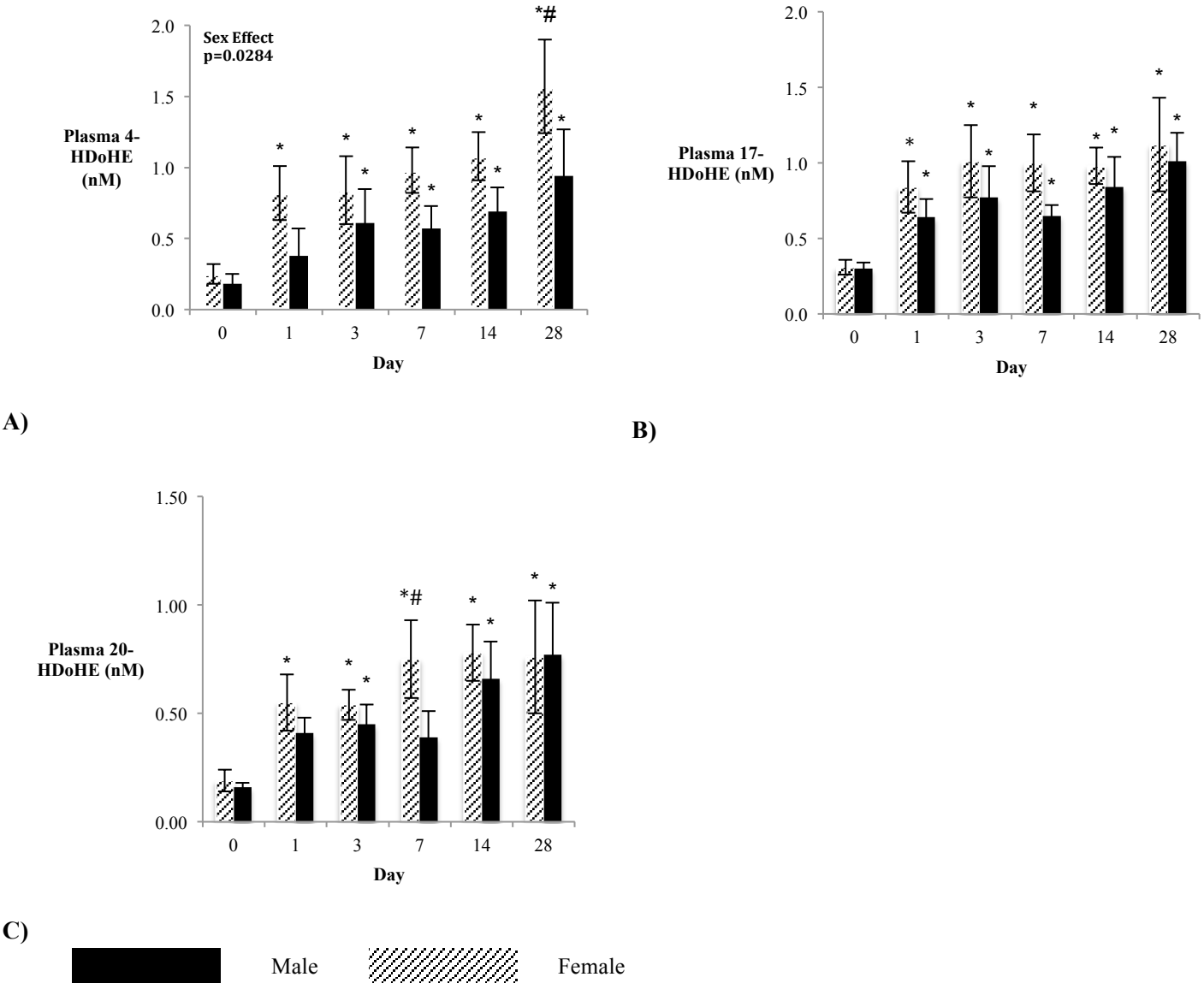


Figure 2-6. Select docosahexaenoic acid derived oxylipins.
Docosahexaenoic acid derived oxylipins representing the pattern observed in plasma from healthy males and females as a function of time and the consumption of fish oil. Sex effect listed where significant. Values represent the mean±SEM. A) 4-HDoHE(4-hydroxydocosahexanoic acid). B) 17-HDoHE (17-hydroxydocosahexanoic acid). C) 20-HDoHE (20-hydroxydocosahexanoic acid). (*) Significant difference between baseline and time point for the same sex (#) Significant difference between sexes for the same time point ($p < 0.05$).

before males (Appendix 4.7 **Table 0-16**).

Oxylipin profiles in serum and plasma at day 28 of omega-3 oil supplementation

Analysis of serum and plasma oxylipins at day 28 of omega-3 oil treatment reveals many alterations between treatments, plasma and serum, and males and females. Of the 73 oxylipins identified in plasma and serum, 46 were significantly altered when looking at treatment, blood type, sex, and the interactions between them (Table 2-5). DHA oil treatment increased several oxylipins including most EPA and DHA derived oxylipins as well as PGD_2 (Table 2-5). AA derived 14,15-DiHETrE was the only analyte that was present in higher quantities in the ALA oil group compared to the DHA oil group. Several oxylipins were more elevated in females when compared to males (i.e. 9-HODE, 13-HODE, 9,10-DiHOME, 18-HEPE, 19,20-DiHDPE) and males had elevated PGD_2, compared to females.

Serum had increased concentrations of several oxylipins as both a main effect as well as within subgroups. Four analytes were increased in serum compared to plasma (TXB_1, PGD_2, 11-HETE, 15-oxoETE) and they are all produced via COX or LOX pathways. Serum oxylipins were further altered when examining sex separately. Males had elevated 2,3-dinor TXB_2, PGD_2, PGE_2, and 11βPGE_2 in serum compared to females, all of which are AA COX derived. Within DHA oil treatment males had increased 12-HHTrE, 15-HETE, 12-oxo-ETE, 20ohLTB_4, LTB_4, and TXB_2 in serum compared to plasma, and females had elevated 10-HDoHE and 11-HDoHE in serum compared to males. These alterations are all observed in oxylipins produced from LOX and COX enzymes. Finally, an increased level of 6k-$PGF_{1\alpha}$, a down stream metabolite of the anti-clotting PGI_2, was observed in plasma.

Table 2-5. Serum and plasma oxylipins for males and females at day 28 of ALA and DHA oil treatment (pM).
Values represent the mean±SEM. Values with similar letters are not different from one another. P<0.05 considered significant.

	ALA				DHA							
	Plasma		Serum		Plasma		Serum					
Oxylipin	Male	Female	Male	Female	Male	Female	Male	Female	Treatment	Blood	Sex	Interaction or Wilcoxon
LA - LOX Oxylipins												
9-HODE	8224 ± 1273	8928 ± 1611	8015 ± 1012	9611 ± 1724	9397 ± 2093	15421 ± 3923	7157 ± 459	12927 ± 2760			0.0277	
13-HODE	4101 ± 568	4720 ± 862	4805 ± 594	5960 ± 1169	4132 ± 685	5748 ± 1112	4268 ± 410	8235 ± 1596			0.0115	
9-oxoODE	124 ± 51	104 ± 35	164 ± 47	212 ± 88	173 ± 60	161 ± 65	128 ± 3.2	239 ± 43				
13-oxoODE	754 ± 195	533 ± 135	1116 ± 270	833 ± 305	340 ± 131	602 ± 267	519 ± 107	1241 ± 531				
9,10,13-TriHOME	8790 ± 2552[a]	4873 ± 1417[a]	4439 ± 2091[a]	2681 ± 425[a]	28674 ± 15112[a]	19588 ± 9942[a]	3163 ± 671[a]	5032 ± 1161[a]				0.0032
9,12,13-TriHOME	10772 ± 3145[a]	5472 ± 1422[a]	5275 ± 1690[a]	1985 ± 517[a]	30965 ± 13979[a]	22215 ± 12580[a]	3196 ± 565[a]	4611 ± 887[a]				0.0007
LA - cP450 Oxylipins												
9,10-diHOME	195 ± 30	280 ± 74	191 ± 30	259 ± 57	196 ± 31	427 ± 109	170 ± 37	295 ± 89			0.0088	
9,10-EpOME	80 ± 33	67 ± 22	51 ± 22	112 ± 41	82 ± 36	73 ± 20	49 ± 21	58 ± 11				
12,13-diHOME	365 ± 124[a]	389 ± 59[a]	347 ± 98[a]	427 ± 54[a]	239 ± 31[a]	555 ± 155[a]	270 ± 31[a]	705 ± 174[a]				0.0393[1]
12,13-EpOME	187 ± 95	272 ± 103	108 ± 28	183 ± 60	105 ± 22	310 ± 101	121 ± 21	170 ± 39				
GLA - LOX Oxylipins												
13-HOTrE-γ	131 ± 29	125 ± 18	73 ± 20	158 ± 28	122 ± 28	101 ± 33	37 ± 26	159 ± 42				
DGLA - COX Oxylipins												
$PGF_{1\alpha}$	275 ± 94	69 ± 10	223 ± 84	90 ± 21	358 ± 105	167 ± 48	124 ± 37	72 ± 30				
TXB_1	--[b]	--[b]	151 ± 25[a]	122 ± 22[a]	--[b]	--[b]	183 ± 16[a]	149 ± 17[a]				<0.0001
DGLA - LOX Oxylipins												
8-HETrE	23 ± 7.7	33 ± 8.2	18 ± 3.7	23 ± 8.4	11 ± 11	26 ± 15	20 ± 12	20 ± 7.7				
15-HETrE	172 ± 29	157 ± 20	167 ± 24	185 ± 27	161 ± 32	177 ± 35	138 ± 35	176 ± 25				
EDA - LOX Oxylipins												
15-oxoEDE	2.5 ± 2.5	1.5 ± 1.3	4.4 ± 1.7	0.7 ± 0.7	0 ± 0	0.8 ± 0.8	0.7 ± 0.7	3 ± 2				
AA - COX Oxylipins												
$11\beta PGE_2$	29 ± 6.7[b]	26 ± 2.9[b]	154 ± 27[a]	70 ± 12[b]	41 ± 4.1[b]	38 ± 5.1[b]	172 ± 18[a]	80 ± 5.9[b]				<0.0001

12-HHTrE	147 ± 123^{b}	44 ± 44^{b}	9854 ± 2935^{ab}	3767 ± 1122^{b}	32 ± 32^{b}	18 ± 18^{b}	17502 ± 6342^{a}	6665 ± 1636^{ab}				<0.0001
2,3-dinorTXB$_2$	0 ± 0^{b}	0 ± 0^{b}	4342 ± 808^{a}	3923 ± 1291^{a}	0 ± 0^{b}	0 ± 0^{b}	4245 ± 1156^{a}	3203 ± 457^{ab}				<0.0001
6k PGF$_{1\alpha}$	13 ± 2.6	5.3 ± 1.3	2 ± 0.7	3.2 ± 2.9	8.2 ± 6.7	6.4 ± 2	5.6 ± 5	3.3 ± 1.5		0.046		
PGD$_2$	16 ± 6.8	16 ± 8.4	55 ± 14	31 ± 6.9	39 ± 13	24 ± 5.8	86 ± 9.1	53 ± 6.6	0.0096	0.0027	<0.0001	
PGE$_2$	20 ± 3.9^{bc}	16 ± 0.8^{c}	99 ± 17^{a}	46 ± 7.1^{bc}	21 ± 6.1^{bc}	23 ± 1.6^{bc}	115 ± 10^{a}	54 ± 4.4^{b}				<0.0001
PGF$_{2\alpha}$	46 ± 14^{a}	33 ± 6.4^{a}	108 ± 45^{a}	40 ± 2.8^{a}	25 ± 4.8^{a}	36 ± 15^{a}	64 ± 3.2^{a}	41 ± 7.7^{a}				0.0137
TXB$_2$	9.2 ± 4.8^{c}	9.4 ± 4.6^{c}	3048 ± 750^{ab}	1639 ± 604^{bc}	34 ± 16^{c}	15 ± 10^{c}	3750 ± 483^{a}	1658 ± 218^{bc}				<0.0001
AA - LOX Oxylipins												
5-HETE	205 ± 31^{b}	287 ± 43^{ab}	469 ± 63^{ab}	491 ± 40^{ab}	227 ± 42^{b}	430 ± 95^{ab}	439 ± 89^{ab}	589 ± 101^{a}				0.0005
8-HETE	304 ± 42	444 ± 51	519 ± 79	612 ± 84	342 ± 76	524 ± 118	451 ± 58	585 ± 89				
9-HETE	237 ± 109	172 ± 68	192 ± 39	194 ± 23	459 ± 311	182 ± 52	585 ± 352	270 ± 35				
11-HETE	298 ± 40	454 ± 87	1599 ± 334	1022 ± 182	419 ± 154	577 ± 153	1838 ± 384	1560 ± 322		<0.0001		
12-HETE	309 ± 60^{b}	483 ± 96^{ab}	9652 ± 3045^{ab}	12125 ± 5665^{a}	458 ± 100^{ab}	697 ± 143^{ab}	10099 ± 2478^{ab}	7417 ± 1939^{ab}				<0.0001
15-HETE	343 ± 50^{c}	386 ± 55^{bc}	705 ± 102^{ab}	623 ± 99^{abc}	369 ± 29^{bc}	425 ± 74^{bc}	794 ± 73^{a}	659 ± 77^{abc}				<0.0001
5-oxoETE	688 ± 592^{a}	1084 ± 1025^{a}	2567 ± 2432^{a}	112 ± 23^{a}	162 ± 61^{a}	158 ± 71^{a}	3407 ± 3215^{a}	157 ± 19^{a}				0.039
12-oxoETE	6.6 ± 3.7^{b}	7.0 ± 1.0^{b}	49 ± 15^{ab}	47 ± 23^{ab}	14 ± 8.6^{b}	8.4 ± 3.3^{b}	84 ± 26^{a}	45 ± 12^{ab}				<0.0001
15-oxoETE	25 ± 3	26 ± 2.8	53 ± 5.6	46 ± 9.5	23 ± 7.1	22 ± 7.3	72 ± 18	49 ± 5.4		<0.0001		
20ohLTB$_4$	0 ± 0^{b}	0 ± 0^{b}	113 ± 52^{ab}	89 ± 23^{ab}	0 ± 0^{b}	0 ± 0^{b}	152 ± 41^{a}	75 ± 33^{ab}				<0.0001
LTB$_4$	3.6 ± 3.6^{bc}	0 ± 0^{c}	51 ± 12^{abc}	53 ± 15^{ab}	0 ± 0^{c}	0 ± 0^{c}	72 ± 25^{a}	45 ± 13^{abc}				<0.0001
LTE$_4$	3.4 ± 3.4^{bc}	0.1 ± 0.1^{c}	49 ± 14^{a}	32 ± 13^{abc}	7.3 ± 4.1^{abc}	1.3 ± 1^{bc}	41 ± 13^{ab}	17 ± 4.5^{abc}				<0.0001
tetranor 12HETE	99 ± 40	117 ± 29	39 ± 7.7	55 ± 12	36 ± 9.2	109 ± 36	40 ± 10	87 ± 24				
AA - cP450 Oxylipins												
5,6-DiHETrE	67 ± 14	56 ± 6.1	124 ± 48	131 ± 24	81 ± 7.9	58 ± 11	70 ± 16	118 ± 24				
5,6-EpETrE	1.2 ± 1.2	3.4 ± 1.8	3.5 ± 1.6	1.1 ± 1.1	0 ± 0	0.9 ± 0.9	1.4 ± 1.4	2.2 ± 1.5				
8,9-DiHETrE	33 ± 3.3	35 ± 3.9	39 ± 3	37 ± 5	27 ± 2.1	29 ± 6.1	27 ± 3.3	38 ± 4.7				
11,12-DiHETrE	135 ± 16	122 ± 11	135 ± 14	138 ± 14	114 ± 14	106 ± 7.4	102 ± 17	125 ± 15				
11,12-EpETrE	9.5 ± 2.3	11 ± 1.5	15 ± 5.9	12 ± 2.7	16 ± 3.4	16 ± 7.6	14 ± 3.4	13 ± 3.1				
14,15-DiHETrE	194 ± 16	186 ± 22	199 ± 22	195 ± 18	153 ± 12	149 ± 5.5	171 ± 18	159 ± 8.6	0.004			
14,15-EpETrE	42 ± 16	50 ± 5.5	70 ± 21	63 ± 21	56 ± 19	53 ± 16	35 ± 6.9	43 ± 14				
16-HETE	183 ± 88	145 ± 30	98 ± 30	139 ± 24	108 ± 15	154 ± 58	63 ± 23	176 ± 73				

17-HETE	53 ± 16	55 ± 10	52 ± 14	43 ± 5.1	39 ± 7.8	49 ± 6.5	51 ± 23	88 ± 34			
18-HETE	89 ± 12^{ab}	85 ± 15^{ab}	76 ± 7^{b}	52 ± 6.2^{b}	101 ± 18^{ab}	134 ± 18^{a}	62 ± 8.3^{b}	74 ± 11^{b}			0.0493[1]
20-HETE	463 ± 56	447 ± 47	492 ± 77	584 ± 83	468 ± 110	622 ± 61	500 ± 58	646 ± 70			
AA - Non-enzymatic Oxylipins											
5-iso $PGF_{2\alpha}VI$	19 ± 4.2	28 ± 8.8	14 ± 4.8	14 ± 5.4	12 ± 7.4	28 ± 10	24 ± 12	28 ± 3			
ALA - LOX Oxylipins											
9-HOTrE	616 ± 267	311 ± 203	448 ± 42	722 ± 79	400 ± 262	266 ± 266	412 ± 112	521 ± 184			
13-HOTrE	737 ± 127	727 ± 67	492 ± 46	823 ± 139	520 ± 138	564 ± 125	400 ± 93	599 ± 176			
9-oxoOTrE	179 ± 70^{a}	42 ± 14^{a}	84 ± 19^{a}	73 ± 24^{a}	99 ± 51^{a}	39 ± 14^{a}	49 ± 21^{a}	98 ± 41^{a}			0.0349[2]
ALA - cP450 Oxylipins											
12,13-diHODE	56 ± 27	146 ± 61	82 ± 34	77 ± 19	39 ± 3.6	136 ± 51	40 ± 16	58 ± 13			
12,13-EpODE	29 ± 8.6	41 ± 8.9	24 ± 6.7	32 ± 10	17 ± 5.1	37 ± 12	21 ± 2.7	34 ± 11			
EPA - COX Oxylipins											
TXB_3	0 ± 0^{b}	0 ± 0^{ab}	24 ± 10^{ab}	16 ± 7.9^{ab}	1.9 ± 1.2^{ab}	0 ± 0^{ab}	45 ± 26^{a}	17 ± 11^{ab}			0.0008
EPA - LOX Oxylipins											
5-HEPE	35 ± 5.3^{c}	82 ± 22^{bc}	82 ± 14^{bc}	107 ± 36^{bc}	103 ± 20^{bc}	201 ± 64^{abc}	209 ± 45^{ab}	346 ± 64^{a}			0.0001
8-HEPE	0 ± 0	1 ± 1	1.2 ± 1.2	1.6 ± 1.6	0 ± 0	0 ± 0	3.8 ± 3.8	0 ± 0			
9-HEPE	68 ± 21	52 ± 12	75 ± 32	93 ± 28	115 ± 48	147 ± 54	154 ± 50	270 ± 50	0.0007		
12-HEPE	65 ± 23^{b}	115 ± 34^{b}	291 ± 123^{ab}	461 ± 160^{ab}	157 ± 32^{ab}	251 ± 25^{ab}	609 ± 170^{a}	617 ± 124^{a}			<0.0001
15-HEPE	86 ± 24^{ab}	80 ± 23^{ab}	55 ± 10^{b}	82 ± 23^{ab}	134 ± 30^{ab}	175 ± 30^{a}	113 ± 32^{ab}	171 ± 20^{a}			0.0036
EPA - cP450 Oxylipins											
18-HEPE	66 ± 13	97 ± 14	83 ± 14	133 ± 21	178 ± 19	256 ± 52	192 ± 30	307 ± 51	<0.0001	0.0011	
DHA - LOX Oxylipins											
4-HDoHE	263 ± 66^{c}	274 ± 79^{c}	273 ± 24^{c}	319 ± 67^{c}	943 ± 305^{abc}	1571 ± 329^{a}	715 ± 94^{bc}	1382 ± 244^{ab}			<0.0001
7-HDoHE	286 ± 143^{a}	208 ± 64^{a}	141 ± 24^{a}	323 ± 111^{a}	429 ± 138^{a}	513 ± 96^{a}	284 ± 74^{a}	589 ± 136^{a}			0.0091
8-HDoHE	203 ± 105^{b}	134 ± 28^{b}	98 ± 18^{b}	147 ± 41^{b}	349 ± 124^{ab}	603 ± 125^{a}	308 ± 57^{ab}	684 ± 129^{a}			<0.0001
10-HDoHE	53 ± 20^{c}	51 ± 13^{c}	64 ± 14^{c}	86 ± 24^{bc}	84 ± 7^{bc}	146 ± 37^{bc}	194 ± 18^{b}	408 ± 61^{a}			<0.0001
11-HDoHE	105 ± 47^{c}	110 ± 34^{c}	117 ± 27^{c}	151 ± 38^{c}	185 ± 5.6^{bc}	376 ± 77^{ab}	253 ± 38^{bc}	496 ± 90^{a}			0.0056[1]
13-HDoHE	38 ± 11^{b}	58 ± 14^{b}	59 ± 13^{b}	72 ± 19^{b}	167 ± 45^{ab}	260 ± 68^{a}	137 ± 18^{ab}	257 ± 66^{a}			<0.0001
14-HDoHE	86 ± 18^{c}	172 ± 40^{c}	937 ± 402^{abc}	1508 ± 610^{abc}	463 ± 147^{bc}	810 ± 174^{abc}	1960 ± 470^{ab}	2315 ± 581^{a}			<0.0001

16-HDoHE	87 ± 14[b]	114 ± 26[b]	84 ± 8.4[b]	111 ± 20[b]	255 ± 50[ab]	396 ± 107[a]	232 ± 46[ab]	348 ± 48[a]			<0.0001
17-HDoHE	304 ± 40	389 ± 50	576 ± 117	360 ± 37	1007 ± 186	1124 ± 309	1018 ± 77	985 ± 239	<0.0001		
DHA - cP450 Oxylipins											
19,20-DiHDPE	854 ± 115	1091 ± 194	780 ± 76	1018 ± 200	1540 ± 201	2153 ± 198	1601 ± 201	2152 ± 231	<0.0001	0.0027	
19,20-EpDPE	30 ± 8.4	28 ± 6.4	53 ± 27	55 ± 25	70 ± 22	62 ± 14	53 ± 23	102 ± 23	0.0367		
20-HDoHE	200 ± 33[b]	186 ± 37[b]	223 ± 51[b]	266 ± 49[b]	771 ± 243[ab]	762 ± 261[ab]	773 ± 260[ab]	966 ± 154[a]			<0.0001

[1]Sex*Treatment interaction; [2]Sex*Blood Interaction; (--) represents analyte was below limit of quantitation

Oxylipin analysis of omega-3 oils

Analysis of the omega-3 oils found high levels of oxylipins in both the ALA and DHA oils. A total of 47 oxylipins were found between the two omega-3 oils including four that were found in DHA oil that were not found in plasma or serum (DHA derived 16,17-EpDPE, EPA derived 14,15-EpETE and 17,18-EpETE, and AA derived 6S-LXA_4) (Table 2-6). ALA oil capsules contained high amounts of 10 LA (5277 mM) and four ALA (1026 mM) oxylipins and also low levels of two AA derived oxylipins (8.9 mM) (Table 2-6). DHA oil capsules contained high levels of EPA (1065 mM) and DHA (1769 mM) derived oxylipins and lower levels of 10 LA (266mM) and 11 AA derived oxylipins (331mM) (Table 2-6). LA derived 9-HODE was found in the largest quantity among all oxylipins present in ALA oil capsules (2527 ± 48 mM) and EPA derived 5-HEPE was found in highest quantities among all oxylipins present in DHA oil (833 ± 95 mM). Finally, ALA oil capsules had a higher concentration of total oxylipins compared to DHA oil (6312 mM compared to 3436 mM).

Table 2-6: Oxylipin concentrations of investigational ALA and DHA oil.
Means of oxylipin analysis of oil capsules in triplicate (mM). Values represent the mean±SEM.

Fatty Acid	Enzyme	Oxylipin	ALA Oil	DHA Oil
LA	LOX	9-HODE	2527 ± 48	24 ± 1.4
LA	LOX	13-HODE	849 ± 55	148 ± 12
LA	LOX	9-oxoODE	898 ± 48	9.0 ± 2.0
LA	LOX	13-oxoODE	822 ± 35	55 ± 6.3
LA	LOX	9,10,13-triHOME	67 ± 5.6	14 ± 14
LA	LOX	9,12,13-triHOME	50 ± 3.7	9.3 ± 9.3
LA	cP450	9,10-diHOME	5.8 ± 0.5	3.0 ± 0.1
LA	cP450	9,10-EpOME	47 ± 4.3	0.5 ± 0.1
LA	cP450	12,13-diHOME	3.4 ± 0.0	2.2 ± 0.1
LA	cP450	12,13-EpOME	8.0 ± 0.4	0.4 ± 0.1
		TOTAL	**5277 mM**	**266 mM**
DGLA	LOX	15-HETrE	--	5.7 ± 0.8
		TOTAL	**--**	**5.7 mM**
AA	COX	$PGF_{2\alpha}$	1.7 ± 0.1	1.9 ± 0.2
AA	LOX	5-HETE	--	175 ± 17
AA	LOX	6S-LXA_4	--	24 ± 4.9
AA	LOX	8-HETE	7.2 ± 0.4	16 ± 2.2
AA	COX/LOX	11-HETE	--	28 ± 4.3
AA	LOX	15-HETE	--	9.1 ± 0.2
AA	cP450	17-HETE	--	21 ± 2.3
AA	cP450	18-HETE	--	9.0 ± 0.1
AA	cP450	5,6-DiHETrE	--	44 ± 7.5
AA	cP450	11,12-EpETrE	--	1.6 ± 0.5
AA	cP450	14,15-DiHETrE	--	1.0 ± 0.1
		TOTAL	**8.9 mM**	**331 mM**
ALA	LOX	9-HOTrE	550 ± 29	--
ALA	LOX	13-HOTrE	261 ± 36	--
ALA	LOX	9-oxoOTrE	148 ± 13	--
ALA	cP450	12,13-EpODE	67 ± 2.4	--
		TOTAL	**1026 mM**	**--**
EPA	LOX	5-HEPE	--	833 ± 95
EPA	LOX	8-HEPE	--	18 ± 2.5
EPA	LOX	9-HEPE	--	46 ± 10
EPA	LOX	12-HEPE	--	45 ± 2.3
EPA	LOX	15-HEPE	--	49 ± 6.6
EPA	cP450	18-HEPE	--	29 ± 2.5
EPA	cP450	14,15-EpETE	--	28 ± 7.1
EPA	cP450	17,18-EpETE	--	17 ± 4.8
		TOTAL	**--**	**1065 mM**

DHA	LOX	4-HDoHE	--	178 ± 29
DHA	LOX	7-HDoHE	--	106 ± 18
DHA	LOX	8-HDoHE	--	190 ± 29
DHA	LOX	10-HDoHE	--	95 ± 20
DHA	LOX	11-HDoHE	--	86 ± 14
DHA	COX/LOX	13-HDoHE	--	148 ± 12
DHA	LOX	14-HDoHE	--	157 ± 13
DHA	LOX	16-HDoHE	--	101 ± 5.3
DHA	LOX	17-HDoHE	--	329 ± 21
DHA	cP450	20-HDoHE	--	76 ± 5.7
DHA	cP450	16,17-EpDPE	--	266 ± 66
DHA	cP450	19,20-EpDPE	--	26 ± 7.2
DHA	cP450	19,20-DiHDPE	--	13 ± 1.4
		TOTAL	**--**	**1769 mM**
		TOTAL OXYLIPINS	**6312 mM**	**3436 mM**

(--) represents analyte was below limit of quantitation

2.5 Discussion

This study is the first of its kind to compare how consumption of 4 g/day of ALA from flax oil with 4 g/day DHA from fish oil over a time course (1, 3, 7, 14, and 28 days) affects the oxylipin profiles of healthy, young males and females. Several alterations in the oxylipin profiles of those consuming DHA oil were observed, with changes beginning as early as at day one of treatment. Further, females responded more quickly to DHA oil treatment and had higher levels of DHA derived oxylipins than males. Fewer alterations were observed in the oxylipin profiles of males and females consuming ALA oil. Several differences were also observed between plasma and serum oxylipin profiles at day 28 of treatment with analytes from several PUFA precursors being elevated in serum. Additionally, oxylipin analysis of the DHA and ALA oils used in this study revealed the presence of several oxylipins at high concentrations. Further, analysis of baseline fatty acid and oxylipin data suggest our wash-in and wash-out periods were sufficient.

Supplementation with flax oil containing ALA and novel DHA enriched fish oil caused several differences in the fatty acid and oxylipin profiles after 28 days of oil treatment. Previous studies have shown increases in plasma EPA and DHA with several weeks of fish oil supplementation leading to increases in several EPA and DHA derived oxylipins [10, 19] and this was also seen with DHA rich fish oil in the present study. However, the same trend was not observed with ALA and ALA derived oxylipins, as ALA oil did not increase total ALA oxylipins after 28 days of treatment. Only one ALA derived oxylipin (13-HOTrE) was elevated from baseline at day 28. These results are somewhat consistent with a study that examined supplementation of 6 g/day of ALA on a younger population where no ALA derived oxylipins had a greater than 2 fold increase after 4 weeks of treatment [18].

Differences between treatments were further seen when examining total omega-3 derived fatty acids and oxylipins. Increases that were observed for total omega-3 fatty acids did translate to increases in total omega-3 oxylipins in the high DHA fish oil group. However, this trend was not observed in the ALA group as no alterations to total omega-3 oxylipins were seen despite an increase in total omega-3 fatty acids. This may be because of a number if reasons including: the contribution of higher ALA in background diet compared to DHA, higher levels of baseline plasma DHA compared to ALA, or higher metabolism of ALA via other processes, such as β-oxidation and storage in adipose [23].

Previous studies have examined two types of fish oil: oil with a higher ratio of EPA to DHA and oils that have approximately equal amounts of EPA and DHA. Higher EPA to DHA oils lead to increases in EPA and DHA derived cP450 oxylipins [9, 10] and hydroxy fatty acids (i.e. 12-HEPE) [9-11] however, when taken in a single dose, no alterations of DHA derived oxylipins are reported [11]. The enriched DHA oil utilized in this study also increased EPA and DHA derived cP450 products, as well as EPA and DHA derived oxylipins produced form COX and LOX pathways, hydroxy fatty acids, including 12-HEPE, and immediately increased EPA and DHA oxylipin production. Fish oils of equal concentration of EPA and DHA increased EPA and DHA derived products at 4 weeks [19], and EPA but not DHA derived products after 12-weeks [12]. The present study elevated both EPA and DHA derived products after 4 weeks of treatment. The novel DHA enriched fish oil used in this study was able to consistently elevate DHA derived oxylipins, something that has not been reported as a result of other fish oils [11, 12]. These results may be due to the increased concentration of DHA present in our supplemental capsules or because previous studies did not look for several of the DHA derived oxylipins that we did in this study.

Retro-conversion of the fatty acids available from DHA oil may have also occurred within participants. Retro-conversion of DHA may occur in males [24], however, in the present study no evidence of retro-conversion of DHA to ALA was observed in males or females, as no significant increase of ALA were observed. Our DHA oil capsules did contain some EPA and so it is unclear if retro-conversion of DHA to EPA occurred within participants.

Further, composition of the DHA enriched fish oil resulted in novel oxylipin profile alterations when compared to fish oil that is equal in concentrations of EPA and DHA or is EPA enriched. Our study identified several DHA derived HDoHEs that have not been reported in other studies supplementing with fish oil including, 4-, 7-, 10-, 11-, 13-, 14-, and 20-HDoHE [9-11]. This may be because previous work has not looked for these oxylipins within their sample. This novel oil further produced rapid increases in most DHA derived oxylipins, which has not been seen with other omega-3 oils that have different ratios of DHA:EPA.

Composition of the ALA and DHA rich oils may have further implications on fatty acid and oxylipin profiles. Fatty acid analysis of investigational DHA oil has shown they contain several other fatty acids beyond EPA and DHA. DPAω3, DPAω6, and AA were present in DHA oil capsules at approximately 2-4% of composition. This may be partially contributing to increases in DPAω6 we observe at several points throughout the time course. Alterations in oxylipin and fatty acid profiles in response to ALA treatment may be due to the composition of the investigational ALA oil. The NOW Flax Oil capsules contained approximately 15% LA per capsule in addition to ALA. This may be the cause for some alterations in plasma LA that were observed in participants, however, there were only minor differences (increased 13-oxoODE at day 7 in females) observed in plasma LA oxylipins.

Supplemental DHA at 4 g/day does appear to be enough to elevate both fatty acid and oxylipin profiles almost immediately. Research that has examined a single dose of fish oil (1008 mg EPA and 672 mg DHA) and the impact on the oxylipin profile over 48 hours shows a very immediate response (after 6 hours) from EPA derived oxylipins and no change in DHA derived oxylipins [11]. We see significant increases in total EPA and DHA derived oxylipins after one day of DHA oil treatment, suggesting our novel DHA enriched oil impacted the profile differently than an oil that contains higher concentrations of EPA. Further, it took 48 hours in some cases for elevated oxylipins to return to baseline levels [11]. Our sample collection was at least 12 hours after participants would have consumed the oil supplements thus making it possible that elevations we saw for some EPA oxylipins were due to recent consumption.

While the ALA dose appears to be sufficient to elevate plasma ALA 2-fold, it had very little impact on the ALA derived oxylipins over the time course. Total ALA oxylipins were not altered over the time course, and only three of the five ALA derived oxylipins found had elevations at some point during the 28-day treatment period. Individual ALA derived oxylipins were found to have variable concentrations throughout the treatment period.

When examining fatty acid and oxylipin profiles separately between sexes several patterns emerge. First, some conversion of ALA was observed in females, but not males, in response to ALA oil treatment. EPA was increased in females at day 7 and 28, however, these increases did not translate into increases in total EPA derived oxylipins (only 5-HEPE at day 7). Increases in plasma DHA were not observed in either sex. In response to ALA oil treatment, ALA incorporation into plasma was different between sexes. Females had increases in ALA at beginning at day one and throughout the treatment phase where as males had increases from

baseline beginning at day 7. No sex differences were observed in incorporation of EPA and DHA in plasma in response to DHA oil treatment.

Oxylipin production in response to DHA oil was different between males and females. Females had higher levels of individual EPA and DHA derived oxylipins compared to males at the same time points. This was seen despite both males and females having increases in plasma DHA beginning at day 1 and throughout the time course. Further, females had increased production from baseline of several DHA and EPA derived oxylipins (HDoHEs and HEPEs) more immediately than males. Females often had alterations from baseline beginning at day one where males tend to show differences from baseline beginning at day 3 or 7. These results suggest that males and females are impacted differently by omega-3 fatty acid consumption, particularly a DHA enriched fish oil. Further, females generally reached a plateau for individual oxylipins earlier than males, particularly in response to DHA oil.

We observed a non-significant trend for females at day 7 of both treatments, with a more pronounced trend observed in ALA oil group. Females displayed elevated, but not significant, total plasma oxylipins, total omega-6 derived oxylipins, and total LOX derived oxylipins at day 7 of treatment. This trend may be explained by hormonal changes that are related to the menstrual cycle. Females began their supplementation phases at 9 ± 2 days after the beginning of menses. This would result in day 7 of the treatment phase to fall at or around the luteinizing hormone surge or the luteal phase of the menstrual cycle, both of which are associated with increase in estradiol and/or progesterone [25]. These hormones are suggested to impact lipid metabolism by increasing DHA concentration in females [2]. However, further research needs to be done to determine the significance of this trend as well as the impact of reproductive hormones on the oxylipin profile.

This was the first time differences within a young healthy population were identified between males and females in plasma and serum oxylipin profiles while consuming omega-3 oil treatments. DHA oil treatment caused increases in several EPA and DHA derived oxylipins as well as PGD_2. In response to ALA oil treatment we see increases in AA derived 14,15-DiHETrE a cP450 product produced by sEH that is associated with decreased blood pressure. This is not consistent with increases in this metabolite seen in previous studies that provide flaxseed to hypertensive patients [17]. Plasma had increased 6k-$PGF_{1\alpha}$, an anti-clotting factor, while serum had elevated clotting factors (i.e. TXB_1 and TXB_2), consistent to what was observed in previous literature [21]. Several sex-specific differences were also identified; including increases in several AA derived oxylipins in males, many from the COX enzymatic pathway. Further, females had increased levels of several LA and DHA derived oxylipins in serum when compared to serum in males as well as plasma in both sexes.

Differences between plasma and serum oxylipin profiles in response to omega-3 oil treatment suggest serum may be a better option when examining oxylipin profiles if the main study objectives aim to investigate blood clotting or to identify and quantitate maximum levels of oxylipins present. Previous research has not directly compared differences in plasma and serum oxylipins between sexes or differences in plasma and serum oxylipins during periods of omega-3 oil supplementation. These results can help us to further understand oxylipin production in healthy young males and females, and may be important when deciding to analyze serum or plasma oxylipins of study participants.

Oxylipin analysis of the investigational product revealed a large concentration of pre-formed oxylipins in both the flax and fish oil capsules. A total of four oxylipins were found in the capsules that were not found in plasma and serum - three DHA derived (cP450 products) and one

AA derived (LOX product). However, there were 30 oxylipins found in plasma that were not in the capsules, the majority being AA derived.

Determining if these oxylipins that are present in supplemental oils have an impact on the oxylipin profiles of individuals is required for us to understand oxylipin production in humans. LA derived 9-HODE was present in ALA oil capsules in the highest concentration of all oxylipins present in the supplemental oils; however, it did not appear to have a significant effect on oxylipin profiles as no differences were seen from baseline in ether sex for this analyte. Further, three DHA derived oxylipins were present in enriched DHA oil capsules and not in human plasma or serum, including 16,17-EpDPE, which was present in the capsules in one of the highest concentration of all DHA oxylipins present. These results suggest the oxylipins present in oils are not impacting circulating oxylipins. However, EPA derived 5-HEPE was found in higher concentrations in the fish oil capsules and was also elevated in plasma, suggesting further studies may be needed to determine how much, if any, pre-formed oxylipins may be absorbed and utilized in the body.

Furthermore, assuming our oil capsules contain approximately 1 g of oil and the average individual has 5 L of blood we can calculate that the highest present oxylipins in flax and fish oils (9-HODE and 5-HEPE) could contribute 3538pM and 1648pM, respectively, per day of supplementation to each liter of blood in the human body. This suggests if the total amount of oxylipins present in the supplemental oils was absorbed, circulating, and not turned over we may see alterations to the oxylipin profiles due to the already present analytes. If all preformed oxylipins from the oil capsules were absorbed the total 9-HODE would be less than half of the total 9-HODE present in human plasma, whereas 5-HEPE present in oil would be present in 10x greater concentrations. Bioavailability of oxylipins is important to investigate, because currently

we cannot distinguish between plasma oxylipins that are absorbed or produced in the body during analysis. Considering the presented data, much is still not understood regarding how consumption of pre-formed oxylipins impacts the oxylipin profile.

2.6 Conclusion

The results from this double-blind randomized controlled study help us to better understand changes that occur during periods of supplementation with 4 g/day of ALA from flax oil and 4 g/day of DHA from fish oil. Females had higher DHA derived oxylipins and displayed increases in DHA derived oxylipins from baseline earlier than males in response to DHA oil treatment. Oxylipins from several PUFA precursors were present in greater quantities in serum and males had several elevated AA oxylipins in serum while females had several higher LA and DHA oxylipins in serum. Oxylipins in high concentrations were also found in both the fish and flax oils. Results from this study will help us to better understand omega-3 oil supplementation in healthy young males and females.

2.7 References for Chapter 2

1. Burdge, G.C. and S.A. Wootton, *Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women.* Br J Nutr, 2002. 88(4): p. 411-20.
2. Giltay, E.J., et al., *Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects.* Am J Clin Nutr, 2004. 80(5): p. 1167-74.
3. Maynar, M., et al., *Menopause-induced changes in lipid fractions and total fatty acids in plasma.* Endocr Res, 2001. 27(3): p. 357-65.
4. Kris-Etherton, P.M., et al., *Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.* Arterioscler Thromb Vasc Biol, 2003. 23(2): p. e20-30.
5. Marik, P.E. and J. Varon, *Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review.* Clin Cardiol, 2009. 32(7): p. 365-72.
6. Mozaffarian, D. and J.H. Wu, *Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events.* J Am Coll Cardiol, 2011. 58(20): p. 2047-67.
7. Fleming, J.A. and P.M. Kris-Etherton, *The evidence for alpha-linolenic acid and cardiovascular disease benefits: Comparisons with eicosapentaenoic acid and docosahexaenoic acid.* Adv Nutr, 2014. 5(6): p. 863S-76S.
8. Gabbs, M., et al., *Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs.* Adv Nutr, 2015. 6(5): p. 513-40.
9. Lundstrom, S.L., et al., *Lipid mediator serum profiles in asthmatics significantly shift following dietary supplementation with omega-3 fatty acids.* Mol Nutr Food Res, 2013. 57(8): p. 1378-89.
10. Nording, M.L., et al., *Individual variation in lipidomic profiles of healthy subjects in response to omega-3 Fatty acids.* PLoS One, 2013. 8(10): p. e76575.
11. Schuchardt, J.P., et al., *Increase of EPA-derived hydroxy, epoxy and dihydroxy fatty acid levels in human plasma after a single dose of long-chain omega-3 PUFA.* Prostaglandins Other Lipid Mediat, 2014. 109-111: p. 23-31.
12. Schebb, N.H., et al., *Comparison of the effects of long-chain omega-3 fatty acid supplementation on plasma levels of free and esterified oxylipins.* Prostaglandins Other Lipid Mediat, 2014. 113-115: p. 21-9.
13. Fischer, R., et al., *Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway.* J Lipid Res, 2014. 55(6): p. 1150-1164.
14. Browning, L.M., et al., *Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish.* Am J Clin Nutr, 2012. 96(4): p. 748-58.
15. *Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA); Panel on Dietetic Products, Nutrition and Allergies*. 2012, European Food Safety Authority: EFSA Journal. p. 2815.
16. Institute of Medicine (U.S.). Panel on Macronutrients and Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. 2005, Washington, D.C.: National Academies Press. xxv, 1-972, 1259-1331 p.

17. Caligiuri, S.P., et al., *Flaxseed consumption reduces blood pressure in patients with hypertension by altering circulating oxylipins via an alpha-linolenic acid-induced inhibition of soluble epoxide hydrolase.* Hypertension, 2014. 64(1): p. 53-9.
18. Caligiuri, S.P., et al., *Elevated levels of pro-inflammatory oxylipins in older subjects are normalized by flaxseed consumption.* Exp Gerontol, 2014. 59: p. 51-7.
19. Keenan, A.H., et al., *Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers.* J Lipid Res, 2012. 53(8): p. 1662-9.
20. Shearer, G.C., et al., *Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters.* J Lipid Res, 2010. 51(8): p. 2074-81.
21. Psychogios, N., et al., *The human serum metabolome.* PLoS One, 2011. 6(2): p. e16957.
22. Quehenberger, O. and E.A. Dennis, *The human plasma lipidome.* N Engl J Med, 2011. 365(19): p. 1812-23.
23. Baker, E.J., et al., *Metabolism and functional effects of plant-derived omega-3 fatty acids in humans.* Prog Lipid Res, 2016. 64: p. 30-56.
24. Schuchardt, J.P., et al., *Effects of docosahexaenoic acid supplementation on PUFA levels in red blood cells and plasma.* Prostaglandins Leukot Essent Fatty Acids, 2016. 115: p. 12-23.
25. Strauss, J.F., III and R.L. Barbieri, *Yen & Jaffe's Reproductive Endocrinology Physiology, Pathophysiology, and Clinical Management*. 2013, Elsevier Health Sciences: London. p. 1 online resource (5646 p.).

3 CHAPTER 3 – THESIS DISCUSSION

The current study examined alterations in the oxylipin profile of young, healthy males and females (n=12) consuming 4 g/day of ALA from flax oil and 4 g/day of DHA from fish oil. This study was a randomized cross-over trial with each supplementation phase lasting four weeks. DHA oil treatment increased plasma fatty acids and oxylipins in individuals in both males and females beginning at day 1 one and throughout the study. However, ALA oil increased plasma ALA in females beginning at day 1 and males at day 7 and throughout the study but caused no changes in total plasma ALA oxylipins for either treatment at any time point.

Females had increased DHA derived oxylipins in response to DHA oil treatment compared to males. Further, females responded more immediately in EPA and DHA oxylipin production over the time course than males consuming DHA oil treatment. Further, several oxylipins from many PUFA were increased in serum over plasma, females were elevated in many of these oxylipins; where males had increases they were more often AA derived. Upon analysis we found both omega-3 treatment oils contained high concentrations of oxylipins. ALA oil contained the highest amount of oxylipin products, including several LA and ALA products, while DHA oil contained several EPA and DHA oxylipins at high concentrations.

3.1 Limitations

While this study had a number of strengths, which are outlined below, there were a few limitations. Participant recruitment was very difficult at the beginning and we found it very difficult to recruit enough participants that fell into our strict BMI, age, and clinical parameter guidelines. Half way through participant recruitment we amended our protocol to widen our parameters to be able to include more participants into our study. BMI was increased from 18-25 kg/m^2 to 18-28 kg/m^2, age was increased from 18-30 to 18-50, plasma creatinine was increased

from normal to <1.5x upper limit of normal where normal is 50-97 umol/L, aspartate transaminase was increased from normal to <2x upper limit of normal where normal is 10-32 U/L, and alanine transaminase was increased from normal to <2x upper limit of normal where normal is <25 U/L. Updating these requirements allowed us to reach our required number of subjects (n=12) while still meeting our guidelines for having a young, healthy population group.

This study would also have benefited from having a control group. Participants could have also consumed an oil containing high monounsaturated fatty acids (i.e. olive oil), as these fatty acids are not known to produce oxylipins. This group could identify if there are fluctuations in oxylipin profiles over time when individuals are not consuming omega-3 oils. Finally, more pure investigational oils may be beneficial for examining alterations in oxylipin profiles. The ALA oil used in the present study had a considerable amount of LA in it and it us unknown how strongly this LA impacted oxylipin production in our participants. Further, the DHA oil used in this study contained some EPA, which limited us from identifying possible retro-conversion of DHA in our healthy, young population.

3.2 Strengths

This double blind randomized control trial has several design related strengths. This is the first study of its kind to examine how supplementation impacts the oxylipin profile over a time course as well as compare plasma and serum oxylipins in males and females after 28 days of omega-3 oil treatment. Additionally, the DHA enriched oil that was used is the first fish oil containing a higher concentration of DHA:EPA to be analyzed for its impact on the oxylipin profile.

The methods of the current study allowed us to scan for 167 oxylipins, while detecting 78 different oxylipins from several PUFA parents including LA, GLA, DGLA, AA, ALA, EPA, and

DHA. Further, this was the first time oxylipins in dietary oil has been examined. Analysis gave us important information about the composition of our investigational products including the possibility that oxylipins within dietary oils may impact the oxylipin profile in plasma/serum.

Sex separation among healthy young individuals when examining oxylipin and fatty acid profiles is critical as it is lacking in the literature. Further, beginning female participants at the same time in their menstrual cycle revealed possible trends in oxylipin production that would not have been identified if females had begun treatments at any time.

3.3 Implications

This study has investigated important information for future clinical work to further look into the differences in oxylipin profiles among those of varying weights and health conditions. We've shown that male and female fatty acid and oxylipin profiles respond almost immediately to supplementation with 4 g/day DHA rich fish oil. However, response to supplementation with ALA rich flax oil on the fatty acid and oxylipin profiles was less clear. We've also shown that there are several differences in fatty acid and oxylipin profiles between sexes consuming DHA rich fish oil and ALA rich flax oil and that work that examines dietary fats and oxylipins should be sex specific.

Examining plasma and serum oxylipin profiles also revealed specific alterations. Many oxylipins were elevated in serum when compared to plasma, suggesting serum may be a better tissue to use when examining oxylipin profiles. Females had several elevated serum oxylipins from LA and DHA when compared to serum in males, however males had several elevated AA derived oxylipins in serum when compared to females. This work provides further information regarding the number of analytes that may be present to quantify in human plasma and serum and will help researchers to select the most appropriate for their work.

Finally, for the first time we identified specific oxylipins that are present in dietary omega-3 flax and fish oils. This work will help other researchers to determine the analytes that may be present in their own supplemental oils. Many aspects of this work will help us to further understand how omega-3 supplementation impacts the body.

3.4 Future Research Directions

With a better understanding of how oxylipin profiles are altered in healthy, young populations during periods of supplementation we can begin to apply this information to different age groups, overweight individuals, and individuals with different health statuses. This could help us to identify possible biomarkers for disease. Further work is needed to investigate possible changes in oxylipin profiles in females in response to the menstrual cycle and hormonal fluctuation. Further, it would be an asset to the field to identify functions of these newly characterized omega-3 oxylipins and to determine if increased production has a benefit, especially in conditions of inflammation. Several omega-3 derived oxylipins have displayed anti-inflammatory properties.

Oxylipins are present in these fish and flax oils at quite large amounts and it could be hypothesized that oxylipins are present in many other PUFA containing oils. Further work to identify oxylipins in dietary oils and how they impact the body utilizing tracer studies could be beneficial to help us understand oxylipins more completely. Finally, the known presence of oxylipins in dietary oils may lead to the production of bioactive nutraceuticals, which would need to be further examined for their effectiveness.

4 CHAPTER 4 – APPENDIX

4.1 Advances in Our Understanding of Oxylipins Derived From Dietary Polyunsaturated Fatty Acids

Melissa Gabbs[1], Shan Leng[1], Jessay G Devassy, Md Monirujjaman, Harold M Aukema*

All authors gave consent to the inclusion of this paper within the present thesis.

Published in Advances in Nutrition 2015:6 513-40

http://advances.nutrition.org/content/6/5/513.abstract

Contributions of Co-Authors

Melissa Gabbs – Co-wrote manuscript, edited manuscript, approved final manuscript.

Shan Leng - Co-wrote manuscript, edited manuscript, approved final manuscript.

Jessay G Devassy – Edited manuscript, approved final manuscript.

Md Monirujjaman, – Edited manuscript, approved final manuscript.

Harold M Aukema – Co-wrote manuscript, Edited manuscript, approved final manuscript.

*Corresponding author: HM Aukema, Human Nutritional Sciences, W573 Duff Roblin Building, University of Manitoba, Winnipeg, MB, Canada R3T 2N2. Email: Aukema@UManitoba.CA

Supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes of Health Research

Conflicts of interest: None

[1]These authors contributed equally to this manuscript

Abbreviations used [follows Lipid Maps format (1, 2)]: AA, arachidonic acid (20:4n-6); AdA, adrenic acid (22:4n-6); ALA, α-linolenic acid (18:3n-3); ASA, acetylsalicylic acid; AT, aspirin-triggered; COX, cyclooxygenase; CYP, cytochrome P450; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid (22:6n-3); DiHDoHE, dihydroxy-docosahexaenoic acid; DiHDPE, dihydroxy-docosapentaenoic acid (DHA metabolite); DiHEDE, dihydroxy-eicosadienoic acid; DiHEPE, dihydroxy-eicosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHODE, dihydroxy-octadecadienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHOTrE, dihydroxy-octadecatrienoic acid; EPA, eicosapentaenoic acid (20:5n-3); EpEDE, epoxy-eicosadienoic acid; EpETrE, epoxy-eicosatrienoic acid (sometimes abbreviated EET); EpETE, epoxy-eicosatetraenoic acid (sometimes abbreviated EEQ); EpDPE, epoxy-docosapentaenoic acid (sometimes abbreviated EDP); EpODE, epoxy-octadecadienoic acid; EpOME, epoxy-octadecenoic acid [also called leukotoxin (9,10 isomer) and isoleukotoxin (12,13 isomer)]; Ex, eoxin; FLAP, 5-lipoxygenase activating protein; GLA, γ-linolenic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; HpDoHE, hydroperoxy-docosahexaenoic acid; HpEPE, hydroperoxy-eicosapentaenoic acid; HpETE, hydroperoxy-eicosatetraenoic acid; HpETrE, hydroperoxy-eicosatrienoic acid; HpODE, hydroperoxy-octadecadienoic acid; HpOTrE, hydroperoxy-octadecatrienoic acid; Hx, hepoxilin; LA, linoleic acid (18:2n-6); LOX, lipoxygenase; Lt, leukotriene; Lx, lipoxin; MaR, maresin; oxo-DoHE, oxo-docosahexaenoic acid; oxo-ETE, oxo-eicosatetraenoic acid; oxo-EPE, oxo-

41 eicosapentaenoic acid; oxo-ODE, oxo-octadecadienoic acid; oxo-OTrE, oxo-octadecatrienoic acid;
42 PD, protectin [also called neuroprotectin (NPD) in the brain]; PG, prostaglandin; PGEM,
43 prostaglandin E metabolite; PMN, polymorphonuclear leukocyte; PPAR, peroxisome proliferator-
44 activated receptor; PUFA, polyunsaturated fatty acid; Rv, resolvin; SDA, stearidonic acid; sEH,
45 soluble epoxide hydrolase; TriHOME, trihydroxy-octadecenoic acid; Trx, trioxilin; Tx,
46 thromboxane

Abstract

Oxylipins formed from polyunsaturated fatty acids (PUFA)[4] are the main mediators of PUFA effects in the body. They are formed via cyclooxygenase, lipoxygenase and cytochrome P450 pathways, resulting in the formation of prostaglandins, thromboxanes, mono-, di- and tri-hydroxy fatty acids, epoxy-fatty acids, lipoxins, eoxins, hepoxilins, resolvins, protectins (also called neuroprotectins in brain) and maresins. In addition to the well-known eicosanoids derived from arachidonic acid, recent developments in lipidomics methodologies have raised the awareness and interest in the large number of oxylipins formed from other PUFA, including those from the essential fatty acids and the longer-chain n-3 PUFA. Oxylipins have essential roles in normal physiology and function, but can also have detrimental effects. Compared to the oxylipins derived from n-3 PUFA, oxylipins from n-6 PUFA generally have greater activity and more inflammatory, vasoconstrictory, and proliferative effects, although there are notable exceptions. As PUFA composition does not necessarily reflect oxylipin composition, comprehensive analysis of the oxylipin profile is necessary to understand the overall physiological effects of PUFA mediated through their oxylipins. These analyses should include oxylipins derived from linoleic and α-linolenic acids, as these largely unexplored bioactive oxylipins constitute more than half of oxylipins present in tissues. Since collated information on oxylipins formed from different PUFA is currently unavailable, this review provides a detailed compilation of the main oxylipins formed from PUFA and describes their functions. Much remains to be elucidated in this emerging field, including the discovery of more oxylipins, and the understanding of the differing biological potencies, kinetics and isomer specific activities of these novel PUFA metabolites.

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. Therefore, since there is currently no collated data on oxylipins in mammalian tissue, the purpose of this review is to provide a detailed compilation of the main oxylipins formed from the various PUFA, and to provide a general overview of their functions.

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. (3-5). 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 ($cPLA_2$) (6). 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 (7, 8). 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 triglyceride lipase in mast cells also reduced oxylipin formation (9). Since triglycerides 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* (10, 11).

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 (12-23).

Cyclooxygenase. The first oxylipin generation pathway involves cyclooxygenase (COX) enzymes, which convert PUFA into prostanoids – i.e. prostaglandins and thromboxanes (12-14). 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 (24, 25). 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) (12, 14). 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 (14). 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] (26-29).

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 (13, 17). An alternative nomenclature is to use the gene names to describe the LOX enzymes (17).

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 (21). 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 (16, 18). Hepoxilins also are formed from 12-HpETE (23) and eoxins from 15-HpETE (30). 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 (13). 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 (13, 15, 19, 20). In addition, they may also enter cells and mediate effects intracellularly by modulating transcription factors and ion channels (15).

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, γ-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 (31, 32), the presence of oxylipins from other PUFA demonstrates that they can be released in sufficient quantities for oxylipin production. Pathways are shown in Figures 1-7 and described by PUFA precursor below.

<u>N-6 PUFA</u>

Arachidonic Acid – Figure 1. AA produces 2-series oxylipins 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 (22). 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).

193 AA also produces oxylipins via the LOX pathway, resulting in HpETE, (e.g. 12-HpETE),
194 which are further rapidly converted to hydroxy fatty acids via glutathione peroxidase (36). 5-, 12-,
195 15-HETE are the most commonly described HETE in mammals, although 8-, 9- and 11-HETE also
196 are produced, and sometimes in greater amounts (37, 38). The 11- or 15-HETE isomers also can be
197 produced via COX activity, as indicated above (26, 27). The HETE can be further converted to
198 oxo-ETE via dehydrogenase activity (39, 40), or to DiHETE, via further COX (e.g. 5,11-DiHETE),
199 LOX (e.g. 5,15-DiHETE) or CYP ω-hydroxylase (e.g. 5,20-DiHETE) activity (41, 42). In addition,
200 the HpETE formed via LOX can be metabolized via several other routes: 5-HpETE can be further
201 converted to 4-series leukotrienes (e.g. LtC_4), via 5-LOX after activation by FLAP; 12-HpETE can
202 be isomerized to hepoxilins (e.g. HxB_3) and subsequently converted to trioxilins [e.g. trioxilin B_3
203 ($TrxB_3$)] (23, 43); and 15-HpETE can be converted to eoxins (e.g. ExC_4) (30). As well, lipoxins
204 (e.g. LxA_4) can be formed from 5- or 15-HpETE via further LOX activity (44-46). Epi-Lx (e.g. 15-
205 epi-LxA_4) formation can also be initiated by aspirin acetylated or nitrosylated COX2 and 5-LOX
206 (47-49). AA also can be converted non-enzymatically to HETE (50) and isoprostanes (e.g. iso-
207 $PGF_{2\alpha}$) (51). The latter are often used as a marker of oxidative stress in vivo; for further discussion
208 of these non-enzymatic oxylipins, see review in (51).
209 AA metabolism via CYP ω-hydroxylase activity results in the formation of HETE with the
210 hydroxy group being at the omega or methyl end of the fatty acid (e.g. 20-HETE), while CYP
211 epoxygenase activity yields epoxy fatty acids (e.g. 14,15-EpETrE), which can be converted to
212 dihydroxy fatty acids (e.g. 14,15-DiHETE), via sEH activity, as reviewed in (15, 19, 20).

213 Formation of other HETE (e.g. 13-HETE) may be mediated via CYP bisallylic hydroxylase activity
214 (52-54), but the importance of this pathway is less known.
215
216 *Linoleic Acid –Figure 2*. Even though the size of the literature for LA oxylipins is markedly
217 smaller than for most other oxylipins (especially AA oxylipins), they are usually present in tissues
218 and blood in higher amounts than oxylipins derived from any other PUFA (55-57). LA produces
219 oxylipins through the LOX pathway, resulting in hydroperoxy fatty acids, which are rapidly
220 converted to hydroxy fatty acids [e.g. 13-hydroxy-octadecadienoic acid (HODE)], which can be
221 further metabolized to keto fatty acids [e.g. 13-oxo-octadecadienoic acid (13-oxo-ODE) (58, 59).
222 LA also can be metabolized via the epoxygenase activity of CYP, resulting in epoxygenated fatty
223 acids [e.g. 9,10-epoxy-octadecenic acid (9,10-EpOME)], which are metabolized via sEH activity to
224 form dihydroxy fatty acids [e.g. 9,10-dihydroxy-octadecenoic acid (9,10-DiHOME)] (60). Further,
225 LA can be converted to trihydroxy fatty acids [e.g. 9,10,13-trihydroxy-octadecenoic acid (9,10,13-
226 TriHOME)] potentially by sequential metabolism of LOX and epoxygenase activity and/or auto-
227 oxidation (61). Several other LA oxylipins also can be produced non-enzymatically (e.g. 9-HODE)
228 (62). There also are reports that the formation of a small amount of the LA oxylipins may be
229 mediated via COX (e.g. 9-HODE) (29, 63) or CYP bisallylic hydroxylation (e.g. 17-HODE) (52-
230 54) activity; the relative importance of these pathways remain to be elucidated.
231
232 *γ-Linolenic Acid.* GLA can be converted via LOX to 10- and 13-hydroxy-octadecatrienoic
233 acid(γ) [13-HOTrE(γ)] (64) 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 (65). Other oxylipins derived from GLA (e.g. 6-HOTrEγ) have been reported to be synthesized in vitro in a patent application (66). Note that oxylipins derived from GLA are distinguished from ALA oxylipins with the use of the γ notation.

Dihomo-γ-Linolenic Acid – Figure 3. DGLA can be converted via COX to 1-series prostaglandins (e.g. PGI_1) and thromboxanes (e.g. TxA_1) (24, 67, 68), via LOX to yield hydroperoxy (e.g. 15-HpETrE) and hydroxy fatty acids [e.g. 15-hydroxy-eicosatrienoic acid (15-HETrE)] (69-74), 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) (70, 71, 75).

Adrenic Acid – Figure 4. AdA can be metabolized by COX into dihomo-prostaglandins such as dihomo-PGE_2, dihomo-TxB_2, and dihomo-PGI_2 (76-81). 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) (78-80), 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) (78).

<u>N-3 PUFA</u>

α-Linolenic Acid – Figure 5. 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)] (82). 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 (29). ALA also can be metabolized via CYP epoxygenase activity, resulting in epoxygenated fatty acids, (e.g. 12,13-EpODE) (65), which can be further converted to dihydroxy fatty acids [e.g. 12,13-dihydroxy-octadecadienoic acid (12,13-DiHODE)] via sEH activity (56). Other ALA metabolites that have been reported include 18-HOTrE from ALA via CYP activity (20), 9,16-DiHOTrE via LOX activity (82) and 12-HOTrE via COX2 activity (29).

Stearidonic Acid. Oxylipins derived from SDA (e.g. 13- hydroxy-octadecatetraenoic acid) have been reported to be produced in vitro in a patent application (66).

Eicosapentaenoic Acid – Figure 6. Similarly to AA, EPA produces oxylipins via the COX pathway, yielding 3-series prostaglandins (e.g. PGE_3) and thromboxanes (e.g. TxA_3) (25). EPA compared to AA is generally a poorer substrate for COX, particularly for the COX1 isoform (83). 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 (25, 84, 85), and 5-series leukotrienes (e.g. LtB_5) via combined 5-LOX and FLAP activity (85, 86). HEPE such as 5-HEPE also can be metabolized to dihydroxy-eicosapentaenoic acids (DiHEPE) such as 5,12-DiHEPE (87) or to keto fatty acids such as 5-oxo-EPE (88). 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) (89, 90). 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(42, 45, 91). EPA can also produce epoxy fatty acids (e.g. 14,15-EpETE) via CYP epoxygenase activity (92), which can be further converted to dihydroxy fatty acids (e.g. 14,15-DiHETE) by sEH (93). As with AA and LA, bisallylic hydroxylation of EPA can also yield HEPE such as 10-HEPE (94).

Docosahexaenoic Acid – Figure 7. DHA can be metabolized via the LOX pathway to hydroxy fatty acids (e.g. 4-HDoHE), with a hydroperoxy intermediate (e.g. 4-HpDoHE) (95). The hydroperoxy 14-HpDoHE can be further metabolized to form maresins (e.g. MaR1) (96), 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 (97) while PDX is formed via double LOX activity (98). 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) (28, 99, 100). DHA also has been shown to yield hydroxy fatty acids non-enzymatically (e.g. 8-HDoHE) (101, 102) and 13-HDoHE can be formed via COX2 (28). Recent studies provide evidence that HDoHE also can be metabolized to dihydroxy (e.g. 14,20-DiHDoHE) (103) and keto fatty acids (e.g. 7-oxo-DoHE) (104) with more likely to be demonstrated in the future. Oxylipins can be produced from DHA via CYP epoxygenase activity,

296 yielding epoxy fatty acids (e.g. 16,17-EpDPE) (92, 95), which can be converted to dihydroxy fatty
297 acids (16,17-DiHDPE) via sEH (93). CYP ω-hydroxylase activity produces HDoHE with hydroxy
298 groups near the methyl end of DHA (e.g. 21-HDoHE) (95).
299
300 **Oxylipin Functions**
301 Oxylipins have a wide range of functions, many of which are still being elucidated. In
302 addition, oxylipins derived from different pathways, as well as different substrate PUFA can have
303 similar or opposing effects, necessitating knowledge of the overall oxylipin profile in order to
304 understand their overall biological effects. Their functions are many, including apoptosis, tissue
305 repair, blood clotting, cell proliferation, blood vessel permeability, pain, inflammation, immune
306 actions and blood pressure regulation (13, 89). General functions of oxylipins are described below
307 and examples of functions are provided in Tables 1-7.
308 N-6 PUFA Oxylipin Functions
309 *COX oxylipins – Tables 1a, 3a, 4a*. The most well known oxylipins are eicosanoids derived
310 from the n-6 PUFA AA. COX derived prostanoids are involved in the regulation of blood pressure,
311 reproduction, diuresis, blood platelet aggregation, modulation of the immune and nervous systems,
312 gastric secretions, cancer, inflammation and the stimulation of smooth muscle contraction, among
313 other effects, as reviewed (12, 14, 340-342). Within these COX metabolites there can be similar
314 and differing effects on these functions. For example, PGI_2 is an anti-aggregatory factor for
315 platelets (343), while TxA_2, serves as a pro-aggregatory factor (344). Another example is the
316 vasodilatory effect of PGI_2 and PGE_2, and the vasoconstrictory effect of $PGF_{2\alpha}$ in some vascular

317 beds (137, 345). PGE_2 also can have effects on thrombosis, which vary depending on the receptor
318 it interacts with. For example, PGE_2 can bind either the EP3 receptor, which makes PGE_2 a pro-
319 thrombotic mediator, or EP4, which makes PGE_2 an anti-thrombotic mediator (346). Similarly,
320 PGD_2 and its metabolites can be both pro-inflammatory and be involved in the resolution of
321 inflammation (34). Compared to COX products formed from AA, those derived from DGLA are
322 usually, but not always less active or produced less efficiently (347). For example, PGE_1 is less
323 stimulatory of aortic smooth muscle cell proliferation than PGE_2 (348). The AdA metabolites,
324 dihomo-PGE_2 and dihomo-PGI_2 also are inactive or much less active compared to their AA
325 analogues with respect to their platelet aggregating activity and contractile properties in both
326 vascular and nonvascular smooth muscle (79, 349).
327
328 *LOX oxylipins – Tables 1b, 2a, 3b*. LOX products such as 5-, 12-, and 15-HETE derived from
329 AA and secreted by epithelial cells and leukocytes are involved in many chronic diseases such as
330 inflammation, obesity, cardiovascular disease, kidney disease and cancer (350-354). As is the case
331 with COX metabolites, AA-derived LOX products can have effects that are both similar to and
332 differing from each other, as well as from those derived via the COX and CYP pathways. For
333 example, 12-HETE has been shown to have both pro- and anti-thrombotic effects (181, 355, 356),
334 while TxA_2 is pro-thrombotic (344) and PGI_2 is anti-thrombotic (343). LOX derived HETE and
335 their oxo-ETE metabolites appear to be primarily pro-inflammatory: for example 5-HETE has
336 chemotactic roles in polymorphonuclear leukocytes (PMN) and rabbit alveolar macrophages (357,
337 358) and stimulates specific granule release from human neutrophils (163). Both 5-oxo-ETE and

12-oxo-ETE also can stimulate eosinophils and neutrophils, but appear to have less activity than their corresponding HETE (156, 359). 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 (360). Eoxins formed from 15-HpETE also have pro-inflammatory effects (30), 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 (197, 198).

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 (189, 190). 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 (16, 44, 361). 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 (362, 363).

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 (364-366), but there are also instances when HODE and their oxo-ODE metabolites are anti-inflammatory and anti-proliferative (178, 273, 367). 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

359 effects, inhibit cancer cell growth and inhibit bleomycin-induced lung fibrosis (368-370), while 15-
360 HETrE has anti-inflammatory effects in skin (273). Three-series leukotrienes derived from DGLA
361 may also reduce inflammation and broncho-constriction due to their relatively lower production
362 compared to 4-series leukotrienes from AA and possibly lower bioactivity (371, 372).
363
364 *CYP Oxylipins – Tables 1c, 2c, 4b.* Oxylipins derived via the CYP pathway from AA include
365 EpETrE and HETE, which have vascular, cardiac and renal functions (15, 373, 374). The effects of
366 these oxylipins also are unique and can be opposing. For example, AA derived EpETrE formed via
367 CYP epoxygenase have hypotensive effects, which is opposite to the hypertensive effects of 20-
368 HETE formed via ω-hydroxylase activity (239, 375). In addition, 16-, 18- and 19-HETE, as well as
369 20-HETE metabolites (20-COOH-AA and 20-OH-PGE_2), also can promote vasodilation (236, 239,
370 376, 377). In some cases, the DiHETrE metabolites of EpETrE formed via sEH activity have less
371 activity (234), but in other cases the DiHETrE have similar or even greater potency (222, 224).
372 Interestingly, sEH inhibitors are currently being used to pharmacologically treat hypertension by
373 prolonging the effects of the epoxy fatty acids on vasodilation (378), but polymorphisms in the
374 CYP enzymes which produce EpETrE do not consistently correlate with effects on hypertension, as
375 reviewed in (379). In addition, EpETrE also play roles in many other biological functions, such as
376 insulin sensitivity (380), hyperalgesia (93) and tumor angiogenesis and metastasis (227, 233).
377 CYP oxylipins formed from LA appear to have similar effects to those derived from AA. For
378 example, 9,10- and 12,13-EpOME derived from LA are produced by neutrophils and macrophages,
379 mediating inflammatory effects (381, 382). 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 (383). Elevated EpOME also has been related to extensive burns, respiratory syndrome and a systemic organ failure in burned skin of humans and lung (384).

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 (385). 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 (288, 386).

COX oxylipins – Table 6a. With respect to COX oxylipins, those derived from EPA are similar to DGLA oxylipins, generally being less potent or are produced less efficiently (288) 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 (385) and demonstrates less mitogenetic and inflammatory activity in fibroblasts and monocytes (282, 385, 387). TxA_3 compared to TxA_2 is produced less efficiently and was reported to have less vasoconstrictory and aggregatory activity (288), 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 (83).

401 PGI_3 and PGI_2 also have similar vasodilatory and anti-aggregatory effects on platelets (288) and
402 TxA_2 and TxA_3 have similar ability to elevate plasma catecholamines in rats, or to activate the TP
403 receptor (83, 285, 288, 386).
404
405 *LOX oxylipins – Tables 5a, 6b, 7a.* LOX also metabolizes the n-3 PUFA, ALA to HOTrE,
406 EPA to HEPE and DHA to HDoHE, oxylipins that also tend to have less inflammatory activity or
407 to be anti-inflammatory. There is very little information on ALA derived oxylipins, but recent
408 findings indicate that 9,16-DiHOTrE has anti-inflammatory and anti-aggregatory effects by
409 reducing prostaglandin production (82), and that 9- and 13- HOTrE are associated with reduced
410 glomerular hypertrophy in obese rats (57). An earlier paper indicates that 13-HOTrE may have
411 anti-inflammatory effects in chondrocytes (275), and a recent paper showed that 13-oxo-OTrE can
412 stimulate glucose uptake and differentiation in adipocytes (277). EPA oxylipins have been much
413 more investigated and are primarily anti-inflammatory; for example, 5-HpEPE can be metabolized
414 to LtB_5, which has less activity, and also competes with LtB_4 and therefore reduces inflammation
415 and broncho-constriction (388-390). 5-oxo-eicosapentaenoic acid (5-oxo-EPE) derived from 5-
416 HEPE is 10-fold less potent in stimulating neutrophils compared with the AA oxylipin (5-oxo-
417 ETE) derived from 5-HETE (88). 15-HEPE derived from EPA also exhibits anti-cancer effects.
418 For example, in human prostatic adenocarcinoma cells 15-HEPE can inhibit cancer cell growth and
419 inhibit production of AA oxylipins (274).
420 DHA also is metabolized via LOX, resulting in the production of HDoHE that also generally
421 exhibit beneficial effects. For example, 4-HDoHE has been reported to inhibit proliferative

retinopathy and retinal endothelial cell proliferation (317) and 14-HDoHE can antagonize platelet activation and smooth muscle constriction (182, 391). 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 (96, 392), or via other DiHDoHE which have similar protective effects, such as the wound healing properties of 14,21-DiHDoHE in mice (315) and inhibition of PMN infiltration in a mouse peritonitis model by 14,20-DiHDoHE (103). Similarly, 17-HDoHE inhibits 5-LOX in rat leukemia cells (84), reduces inflammation and oxidative damage in murine hepatocyte injury (318) and has anti-hyperalgesic properties in a rat model of arthritis (320). 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 (99, 393, 394), while the effects of protectins vary by isomer – PDX has anti-aggregatory effects (328, 395) and can restore insulin sensitivity in obese mice (331), but PD1 does not exhibit these activities (331, 396). Both can inhibit influenza virus replication (397, 398), reduce inflammation and accelerate the resolution of inflammation (394), 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 (97, 399).

CYP oxylipins – Tables 5b, 6c, 7b. 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

443 EpETrE in some vascular beds (339, 400). In addition, several CYP isoforms preferentially
444 metabolize n-3 over n-6 PUFA, as reviewed in (89, 401). EpETE can also inhibit Ca^{2+} and
445 isoproterenol induced contractility of neonatal cardiomyocytes, suggesting they have
446 antiarrhythmic effects (402). EpDPE derived from DHA has anti-inflammatory, vasodilatory and
447 anti-cancer effects, similar to EpETE (233, 301, 339). EpDPE also can inhibit angiogenesis and
448 metastasis (233), unlike the AA derived EpETrE, which promote these functions (227). 18-HEPE
449 derived from EPA via ω-hydroxylase also appears to have an anti-cancer role by down regulating
450 pro-inflammatory and pro-proliferative factors (306), possibly via conversion to E-series resolvins.
451 These resolvins have similar effects as the D-series resolvins, markedly reducing PMN infiltration,
452 decreasing pro-inflammatory cytokines, and enhancing the resolution of inflammation (361, 403,
453 404).
454
455 In summary, oxylipins have important biological effects that mediate normal physiology and
456 function. However, compared to oxylipins derived from n-3 PUFA, those derived from n-6 PUFA
457 have more inflammatory, vasoconstrictory, and proliferative effects, with the exception of several
458 examples, such as some prostanoids and/or their metabolites, lipoxins, some oxylipins from DGLA
459 and LA, EpETrE and some CYP derived HETE. On the other hand, most oxylipins derived from n-
460 3 PUFA tend to have less activity or be anti-inflammatory, pro-resolving, vasodilatory, and anti-
461 proliferative. In addition, some of the anti-inflammatory and vasodilatory CYP oxylipins derived
462 from EPA and DHA have even greater potency than their AA counterparts.
463

Future Developments in Nutrition and Oxylipin Research

Given the vastly differing and often opposing functions, it is critical that comprehensive analyses of the oxylipin profile are performed in order to gain an overall understanding of the biological effects. To date, few studies have examined the whole range of PUFA derived oxylipins, but the recent development of mass spectrometry based methods is enabling this possibility (405). The number of oxylipins being measured by these methods continues to grow – e.g. novel protectin and maresin like products from both the n-3 and n-6 docosapentaenoic acid isomers (97, 399). Recently, several reports have described the oxylipin profile in human blood (55, 406) and a small number of studies have examined the serum oxylipin profile of in response to fish oil supplementation in healthy individuals (407-410) as well as those who have asthma (411). These analyses and other studies that have increased dietary LA or ALA have revealed that the type of dietary fat significantly alters oxylipin profiles (57, 412-414). Furthermore, these studies have demonstrated that the oxylipins derived from LA and ALA make up more than half of the total oxylipins content measured. Despite this, much less is known about these oxylipins and future studies characterizing the levels, as well as determining their biological activities will greatly increase our understanding of the effects of nutritional interventions in health and disease.

In this regard, there are some studies that have examined oxylipin activities side-by-side, such as those derived from EPA or DHA compared to those derived from AA (see Tables 6 & 7), which generally, but not always, exhibit lesser activity in the former than the latter. However, comparisons of the biopotencies of most of the LA and ALA oxylipins are unknown, either to each other, or to their elongation counterparts. These, and studies that examine the relative biological

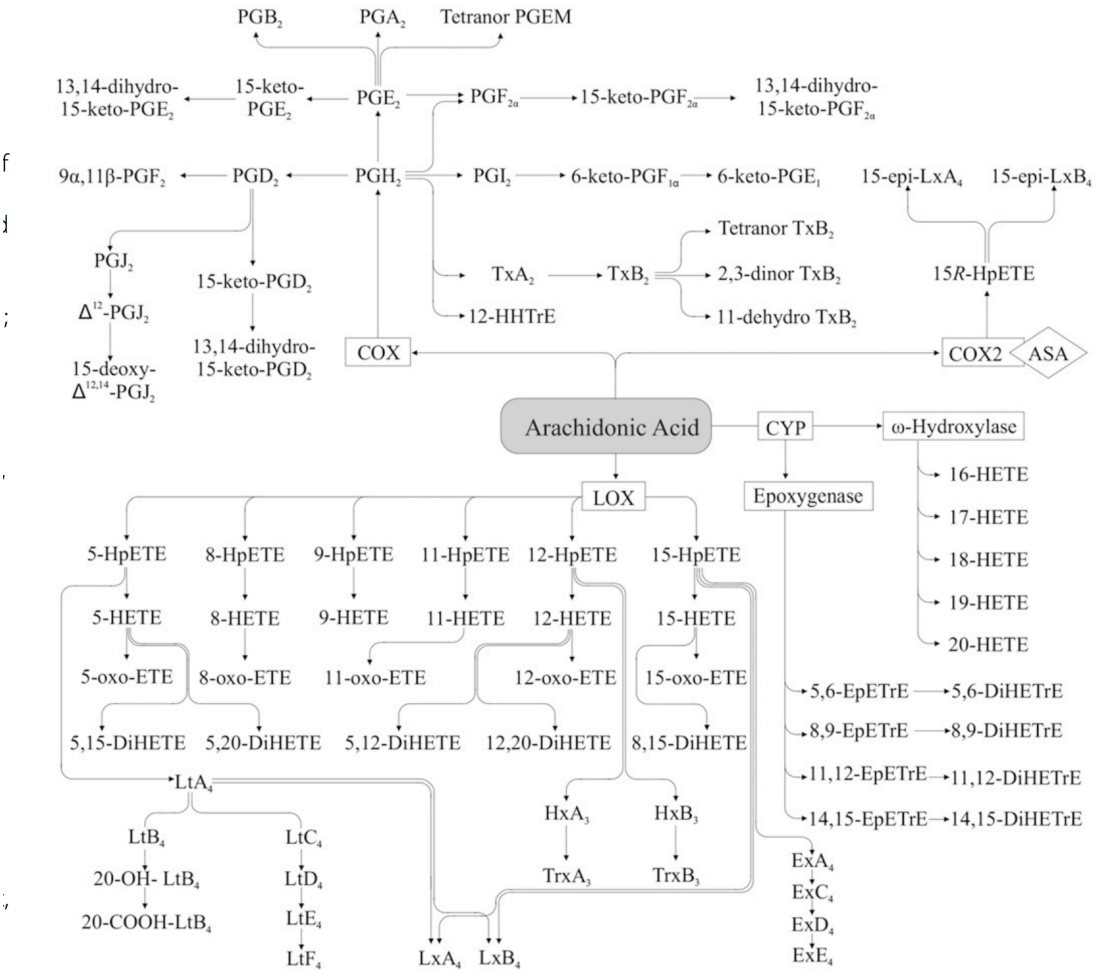
485 activities of oxylipins are needed in order to further our understanding of the physiological effects
486 of the entire oxylipin profile. In addition, while some studies have compared the effects of oxylipin
487 stereoisomers, much more knowledge in this area also is required. Differentiation between enzyme
488 mediated and autooxidation products and their potential effects in biology will also be facilitated by
489 these studies.

490 It is important to note that tissue PUFA composition cannot be used to reliably predict the
491 oxylipin content of tissues, despite that this has routinely been done in the past literature. This was
492 illustrated in a recent targeted lipidomic analysis of renal oxylipins in obese rats, which
493 demonstrated that while the PUFA content generally reflected oxylipin content, there were notable
494 discrepancies. For example, with 9-fold differences in the amounts of LA in the diets of these rats,
495 the AA content of the renal phospholipid was the same, but the levels of several AA derived
496 oxylipins were different (57). This has important implications for the current debate surrounding
497 the dietary recommendations for LA (415). Furthermore, this study indicated that PUFA
498 conversion to oxylipins varies by as much as 10-fold between PUFA, with ALA being metabolized
499 to oxylipins at a greater rate than LA, AA or EPA, for example. This may be due to differences in
500 incorporation and release of phospholipid fatty acid, as well as differences in conversion to
501 metabolites, which may be less, more or equally active. ALA also increased the level of oxylipins
502 derived from EPA and DHA even though no EPA or DHA was present in the diets, demonstrating
503 that PUFA also may mediate some of their effects via oxylipins derived from PUFA formed via
504 elongation and desaturation of the shorter PUFA (57). Hence, there also is a need for kinetic
505 analysis of oxylipin formation and turnover [also referred to as fluxolipidomics (416, 417)], which

506 also will improve our understanding of the physiological effects of oxylipins in vivo.
507 Comprehensive analyses that include the LA and ALA oxylipins in differing tissues in response to
508 dietary interventions promises to yield significant novel information on the large numbers of these
509 bioactive compounds.

Figure Legends

Figure 1. AA derived oxylipins.



Abbreviations: ASA, acetylsalicylic acid; COX, cyclooxygenase; CYP, cytochrome P450; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; EpETrE, epoxy-eicosatrienoic acid (sometimes abbreviated EET); Ex, eoxin; HETE, hydroxy-eicosatetraenoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HpETE, hydroperoxy-eicosatetraenoic acid; Hx, hepoxilin; LOX, lipoxygenase; Lt, Leukotriene; Lx, lipoxin; oxo-ETE,

518 oxo-eicosatetraenoic acid; PG, prostaglandin; PGEM, prostaglandin E metabolite; Trx, trioxilin;

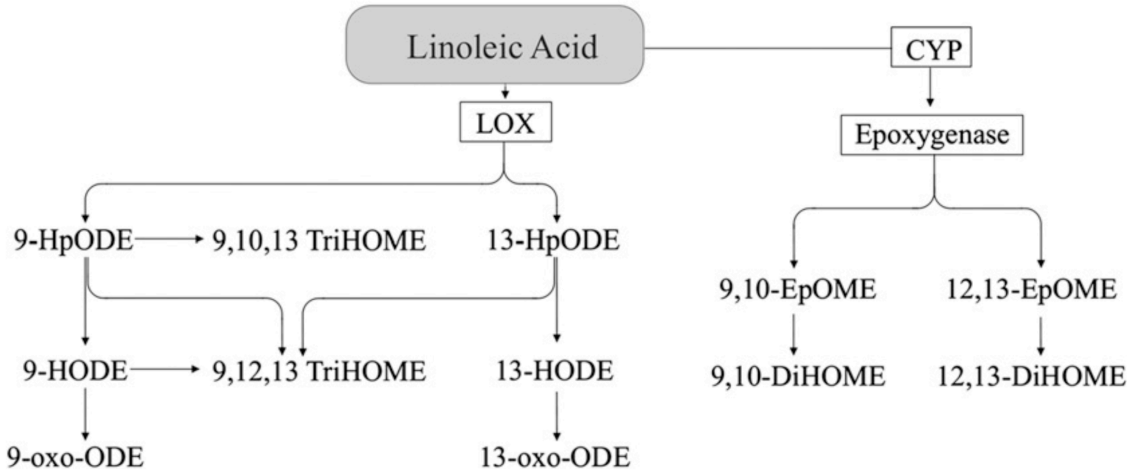
519 Tx, thromboxane

520 [a]There is also evidence for thromboxane synthase-independent production of HHTrE (418)

521 [b]Also produced via the COX pathway (26, 27)

522

523 Figure 2. LA derived oxylipins.



524

525 Abbreviations: CYP, cytochrome P450; DiHOME, dihydroxy-octadecenoic acid; EpOME, epoxy-

526 octadecenoic acid [also called leukotoxin (9,10 isomer) and isoleukotoxin (12,13 isomer)]; HODE,

527 hydroxy-octadecadienoic acid; HpODE, hydroperoxy-octadecadienoic acid; LOX, lipoxygenase;

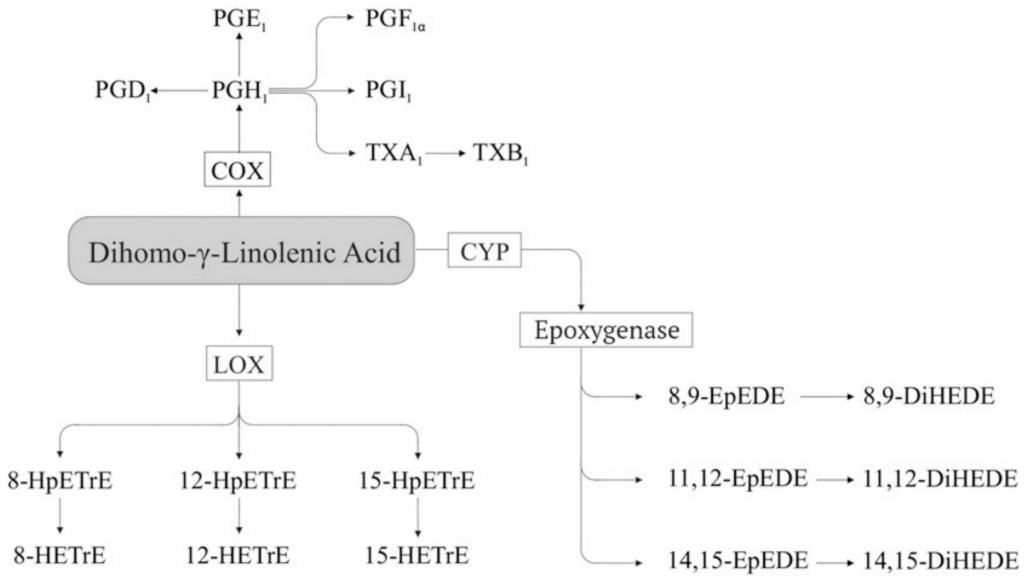
528 oxo-ODE, oxo-octadecadienoic acid; TriHOME, trihydroxy-octadecenoic acid

529 [a]Also produced via the COX pathway (29, 63)

530

531

532 Figure 3. DGLA derived oxylipins.



533

534 Abbreviations: COX, cyclooxygenase; CYP, cytochrome P450; DiHEDE, dihydroxy-eicosadienoic

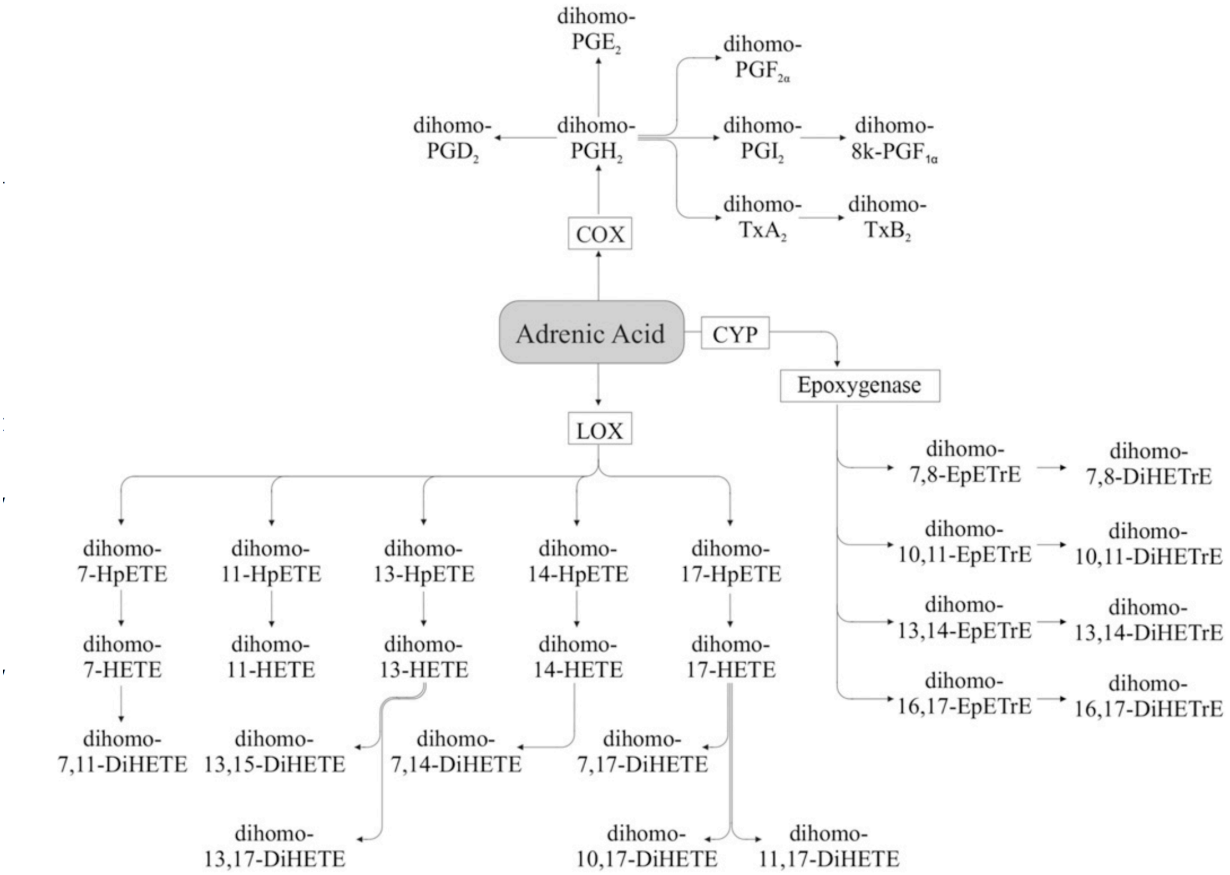
535 acid; EpEDE, epoxy-eicosadienoic acid; HETrE, hydroxy-eicosatrienoic acid; HpETrE,

536 hydroperoxy-eicosatrienoic acid; LOX, lipoxygenase; PG, prostaglandin; Tx, thromboxane

537

538

539 Figure 4. AdA derived oxylipins.



540

541 Abbreviations: COX, cyclooxygenase; CYP, cytochrome P450; DiHETE, dihydroxy-

542 eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; EpETrE, epoxy-eicosatrienoic acid

543 (sometimes abbreviated EET); HETE, hydroxy-eicosatetraenoic acid; HpETE, hydroperoxy-

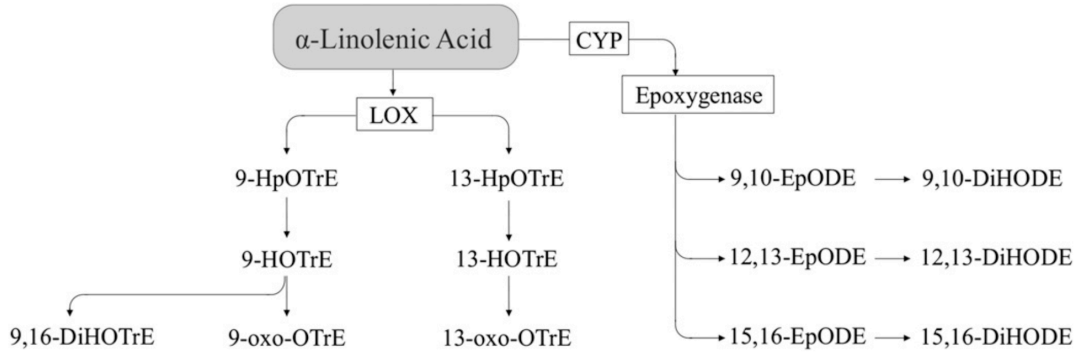
544 eicosatetraenoic acid; LOX, lipoxygenase; PG, prostaglandin; Tx, thromboxane

545 [a]Also can be formed from dihomo-7-HETE (78)

546

547

548 Figure 5. ALA derived oxylipins.



549

550 Abbreviations: CYP, cytochrome P450; DiHODE, dihydroxy-octadecadienoic acid; DiHOTrE,

551 dihydroxy-octadecatrienoic acid; EpODE, epoxy-octadecadienoic acid; HOTrE, hydroxy-

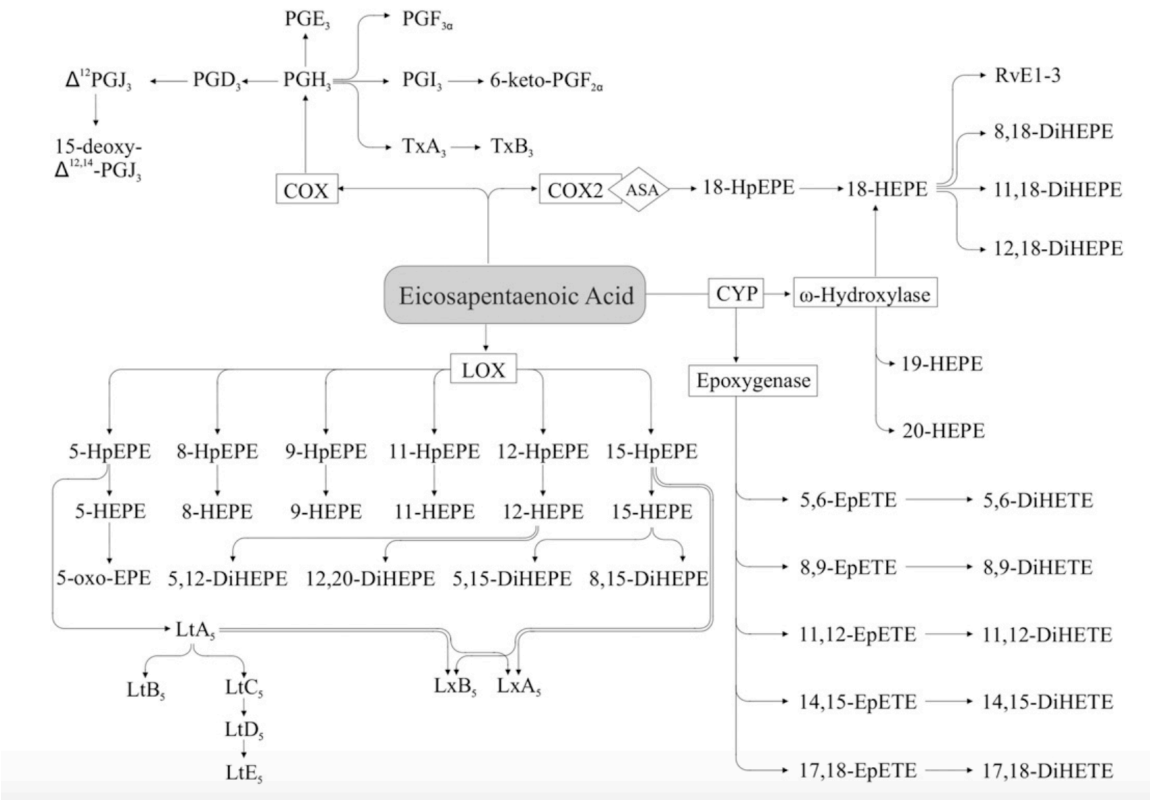
552 octadecatrienoic acid; HpOTrE, hydroperoxy-octadecatrienoic acid; LOX, lipoxygenase; oxo-

553 OTrE, oxo-octadecatrienoic acid

554

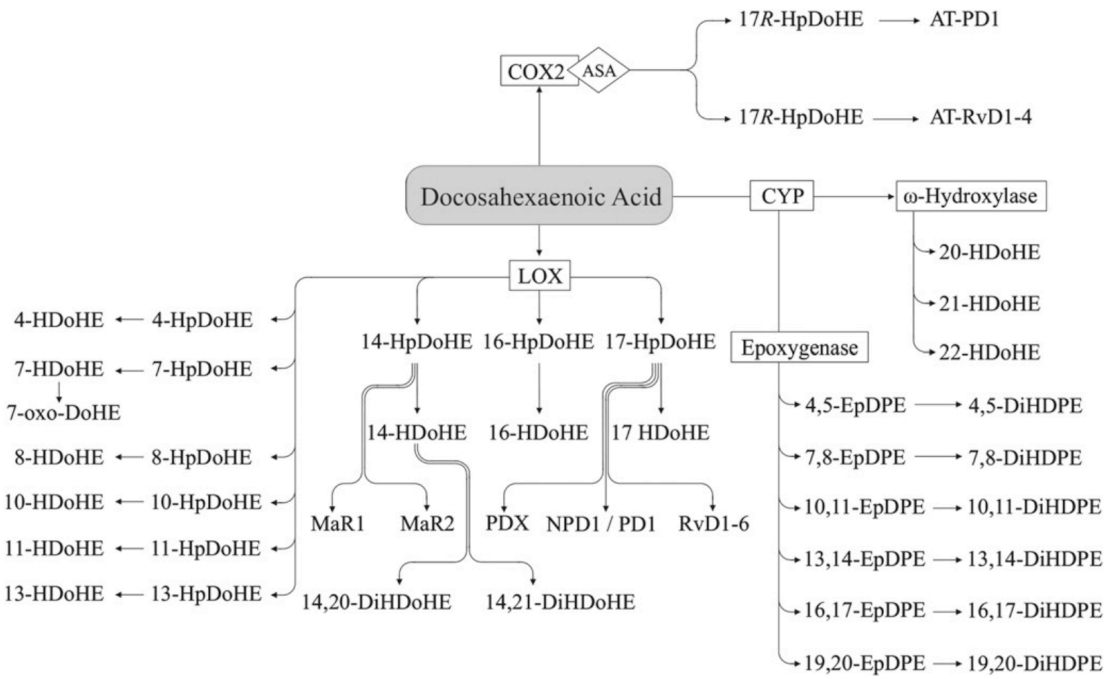
555

Figure 6. EPA derived oxylipins.



Abbreviations: ASA, acetylsalicylic acid; COX, cyclooxygenase; CYP, cytochrome P450; DiHEPE, dihydroxy-eicosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; EpETE, epoxy-eicosatetraenoic acid (sometimes abbreviated EEQ); HEPE, hydroxy-eicosapentaenoic acid; HpEPE, hydroperoxy-eicosapentaenoic acid; LOX, lipoxygenase; Lt, Leukotriene; Lx, lipoxin; oxo-EPE, oxo-eicosapentaenoic acid; PG, prostaglandin; Rv, resolvin; Tx, thromboxane

565 Figure 7. DHA derived oxylipins.



566

567 Abbreviations: ASA, acetylsalicylic acid; AT, aspirin-triggered; COX, cyclooxygenase; CYP,

568 cytochrome P450; DiHDoHE, dihydroxy-docosahexaenoic acid; DiHDPE, dihydroxy-

569 docosapentaenoic acid; EpDPE, epoxy-docosapentaenoic acid (sometimes abbreviated EDP);

570 HDoHE, hydroxy-docosahexaenoic acid; HpDoHE, hydroperoxy-docosahexaenoic acid; LOX,

571 lipoxygenase; MaR, maresin; oxo-DoHE, oxo-docosahexaenoic acid; PD, protectin [also called

572 neuroprotectin (NPD) in the brain]; Rv, resolvin;

573 [a]Also produced via the COX pathway (28)

574 [b]Also may be formed from 21-HdoHE (315, 316)

Table 1. Examples of AA derived oxylipin functions

A. COX oxylipins	
PGA_2	Contributes along with PGE_2 to the development of Th1-type immune responses, with PGE_2 being more potent in human monocyte derived dendritic cells (105)
	Inhibits Ca^{2+}-stimulated ATPase activity of Walker-256 tumor microsomal membranes (106)
	Represses insulin-like growth factor-I gene expression in C6 rat glioma cells (107)
PGB_2	Mediates mesenteric vascular dose-dependent vasodilatory and vasoconstrictory effects in animal models (108)
	Elevates blood pressure, tracheal segment pressure and bronchial resistance in guinea pigs (109)
PGD_2	Inhibits induced apoptosis in human articular chondrocytes (110)
	Inhibits murine lung inflammation (111)
	Promotes sleeping behavior (112)
	Regulates body temperature in rodent models (113, 114)
	Inhibits tumor cell proliferation in human cells and rodent model (115)
	Pro-inflammatory at nanomolar concentrations and anti-inflammatory at micromolar concentrations [reviewed in (116)]
	Inhibits human neutrophil activation in vitro (117, 118)
	Causes apoptosis of human eosinophils (119)
	Activates human eosinophils (120)
	Inhibits human platelet aggregation (121, 122)
PGE_2	Vasodilates cat cerebral arterioles (123)
	Potentiates human platelet aggregation at lower concentrations and inhibits aggregation at a higher concentrations (124)
	Induces human colon cancer cell growth (125)
	Stimulates IL-10 production in bone marrow-derived dendritic cells in murine model (126)
	Mediates lung inflammation in human cells (127)
15-keto-PGE_2	Activates PPARγ to enhance adipogenesis of murine 3T3-L1 cells (128)
6-keto-$PGF_{1\alpha}$	Stable degradation product of PGI_2 and useful marker of PGI_2 in humans (129, 130)
9α,11β-PGF_2	Activates murine eosinophils (131)
$PGF_{2\alpha}$	Mediates inflammatory tachycardia in the mouse (132)
	Initiates parturition in the mouse (133)
	Vasoconstricts rat brain arterioles (134)
13,14-dihydro-15-keto-$PGF_{2\alpha}$	Reflects in vitro $PGF_{2\alpha}$ biosynthesis and is the main inactive degradation product of $PGF_{2\alpha}$ in humans (135)
PGI_2	Inhibits ADP-induced hamster platelet aggregation in (136)
	Induces coronary vasodilation in dogs (137)
	Inhibits adhesion of human eosinophils to lung endothelial monolayers and transendothelial migration (138)
	Inhibits erythrocyte adhesion to bovine aortic endothelial cells (139)
PGJ_2	Causes apoptosis of human eosinophils (119)
	Induces respiratory burst in human eosinophils (120)
Δ^{12}-PGJ_2	Releases eosinophils from guinea pig bone marrow and induces respiratory burst in human eosinophils (120)
	Causes apoptosis of human eosinophils and neutrophils (119)
15-deoxy-$\Delta^{12,14}$-PGJ_2	Inhibits induced apoptosis in human articular chondrocytes (110)
	Anti-inflammatory via inhibition of NF-κB activation in human and monkey cell culture (140)
	Causes apoptosis of human eosinophils and neutrophils (119)

	Induces respiratory burst in human eosinophils (120)
	Reduces the apoptosis in activated human and murine T-lymphocytes (141)
TxA_2	Mediates inflammatory tachycardia in the mouse (132)
	Causes irreversible platelet aggregation in human platelet rich plasma (142)
	Stimulates mitogenesis of coronary artery smooth muscle cells in guinea pig model (143)
	Mediates hypertension in hypertensive rats (144)
	Vasoconstricts rabbit aorta (145)
TxB_2	Has a weak bronchoactive effect in guinea pigs and dogs (146)
	Increases systemic vascular resistance but does not cause platelet aggregation in dogs (147)
	Chemotactic in human peripheral PMN (148)
2,3-dinor-TxB_2	Marker of thromboxane synthesis in urine of rats (149, 150)
	Possible urinary marker of acute myocardial infarction in humans (151)
11-dehydro-TxB_2	Plasma and urinary marker of thromboxane synthesis in human and rabbit models (152-154)
	Possible urinary marker of acute myocardial infarction in humans (151)
B. LOX oxylipins	
5,15-DiHETE	Possesses weak human neutrophil and eosinophil chemotactic activity (155, 156)
8,15-DiHETE	Possesses weak human eosinophil chemotactic activity (155)
	Exhibits chemotactic activity comparable to that of LtB_4 for human PMN (157)
12,20-DiHETE	Activates cholesterol ester hydrolysis in human vasculature (158)
Eoxins	Eoxin C_4, D_4 and E_4 all increase permeability of endothelial cell monolayer from human eosinophils and mast cells in vitro (30)
5-HETE	Inhibits the clonal proliferation of chick embryo fibroblasts and granulocytic progenitors (159)
	Stimulates human eosinophil chemotaxis and chemokinesis (160)
	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (161, 162)
	Induces human neutrophil degranulation (163)
	Inhibits PGI_2 production in porcine coronary artery endothelial cells (164)
	Inhibit selenium-induced apoptosis in human prostate cancer cells; 12- and 15-HETE have no effect (165)
	Stimulates proliferation of human cancer cells at low concentrations (166)
	Promotes bovine neutrophil chemotaxis in vitro more potently than 5-HEPE (167)
5-HpETE	Inhibits human platelet aggregation similarly to 5-HpEPE, but less potently than 12- or 15-HpETE (168)
5-oxo-ETE	Stimulates human neutrophils and eosinophils (88, 169)
	Inhibits selenium-induced apoptosis in human prostate cancer cells, with half the potency of 5-HETE (165)
	Stimulates proliferation of human cancer cells in low concentrations and inhibits proliferation at higher concentrations (166)
	Promotes chemotaxis and raises cytosolic calcium levels in human neutrophils; more potent than 5-HETE, 15-oxo-ETE and 5,15-DiHETE (156)
	Stimulates human neutrophils more potently than 5-HETE (170)
	Does not inhibit LOX enzyme activity (cf. 12- and 15-oxo-ETE) in vitro (171)
8-HETE	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (161)
	Promotes wound healing via epithelial cell migration in rat cornea (38)
	Induces differentiation of murine 3T3-L1 pre-adipocytes (172)
9-HETE	Stimulates human eosinophil chemotaxis and chemokinesis (160)
	Stimulates human neutrophil chemokinesis and enhances chemotactic responses

	(161)
11-HETE	Stimulates human eosinophil chemotaxis and chemokinesis (160) Stimulates human neutrophil chemokinesis and enhances chemotactic responses (161, 162) Inhibits human vascular smooth muscle cell proliferation (173)
11-oxo-ETE	Inhibits human colorectal adenocarcinoma epithelial and umbilical vein endothelial cell proliferation in culture (174)
12-HETE	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (161) Induces human neutrophil degranulation (163) Increases rat heart mitochondrial calcium and nitric oxide, leading to oxidative stress and apoptosis (175) Increases monocyte adhesion to human endothelial cells leading to aortic fatty streak formation (176, 177) Enhances tumor cell adhesion to endothelial cells in mice (178) Enhances thrombin-induced aggregation (179), but suppresses collagen-induced aggregation of bovine platelets (180) Inhibits U-46619-induced aggregation of human platelets (181, 182) Reduces ADP-induced aggregation of mouse platelets (183) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (139)
12-HpETE	Inhibits human platelet aggregation similarly to 12-HpEPE, and more potently than 5- or 15-HpETE (168, 184)
12-oxo-ETE	Selectively inhibits LOX enzyme activity in vitro (171) Activates human neutrophils (185)
15-HETE	Exhibits vasodilation or vasoconstriction in isolated arteries from guinea pig, rabbit, rat and human depending on species and conditions (186) Activates $PPAR_{\Upsilon}$ in human and $PPAR_{\beta/\delta}$ in mouse (187, 188) Inhibits human PMN migration across cytokine-activated endothelium in vitro (189) Inhibits degranulation and superoxide production in stimulated human PMN (190) Mediates hypoxia-induced rabbit pulmonary hypertension (191) Enhances thrombin-induced human platelet aggregation (192) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (139)
15-HpETE	Exhibits vasodilation or vasoconstriction in isolated arteries from guinea pig, rabbit, rat and human depending on species and conditions (186) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (139) Induces migration of monocyte-like HL-60 cells across a human endothelial cell monolayer (193) Induces loss of rat cardiomyocyte membrane integrity (194) Inhibits human platelet aggregation similarly to 15-HpEPE, but less potently than 12-HpETE (168)
15-oxo-ETE	Selectively inhibits human LOX enzyme activity in vitro (171) Inhibits human vascular vein endothelial cell proliferation (195) Prevents apoptosis of rat pulmonary arterial smooth muscle cells (196)
HxA_3	Activates human neutrophils (197) Recruits human PMN to the site of inflammation (198) Promotes murine 3T3-L1 preadipocyte differentiation (199)
HxB_3	Promotes murine 3T3-L1 preadipocyte differentiation (199)
LtB_4	Releases human PMN lysosomal enzymes (200) Induces human PMN chemotaxis and aggregation (201, 202) Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (203) Promotes chemotaxis of bovine neutrophils, more potently than LtB_5 (167)
20-OH-LtB_4	Stimulates human neutrophil migration, but less potently than LtB_4 (204) Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (203)

20-COOH-LtB_4	Stimulates human neutrophil migration, but less potently than LtB_4 (204)
LtB_4	Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (203)
LtC_4	Causes guinea pig uterine and lung contractions (205) Stimulates guinea pig lung strip contraction, more potently than LtB_4 (203) Mediates human skin inflammation (206) Increases permeability of endothelial cell monolayers from human eosinophils and mast cells in vitro (30) Contracts guinea pig lung parenchymal strips and ileal tissues, with similar potency to LtC_5 (207)
LtD_4	Enhances responsiveness to histamine in bovine airway smooth muscle (208) Causes guinea pig uterine and lung contraction (205) Mediates human skin inflammation (206) Increases permeability of endothelial cell monolayer monolayers from human eosinophils and mast cells in vitro (30)
LtE_4	Causes guinea pig uterine and lung contraction (205) Coronary constrictor in the in situ pig heart (209)
LtF_4	Induces bronchoconstriction in guinea pig, but less active than LtD_4 (210)
LxA_4	Inhibits LtB_4-induced human PMN activation (211) Stimulates human monocyte migration and adhesion (212) Inhibits zymosan A-induced peritonitis in mice (213) Promotes corneal epithelial cell wound healing in mice (214) Increases renal plasma flow and glomerular filtration rate in the rat (215) Stimulates phospholipid remodeling without causing aggregation in human neutrophils (216) Antagonizes LtD_4-induced lowering of glomerular filtration rate in rat (217) Induces contraction of isolated guinea pig pulmonary smooth muscle (similar to LxA_5 and LxB_4 effects), and vasorelaxation of rat or guinea pig aortic rings (similar to LxB_4) (218) Inhibits proliferation of human A549 cells, but less potently than 15-epi LxA_4, 15-epi LxB_4 or LxB_4 (47)
LxB_4	Stimulates human monocyte migration and adhesion (212) Decreases renal plasma flow and glomerular filtration rate in the rat (215) Inhibits zymosan A-induced peritonitis in mice (213) Stimulates phospholipid remodeling without causing aggregation in human neutrophils (216) Induces contraction of isolated guinea pig pulmonary smooth muscle (similar to LxA_4 and LxA_5 effects), and vasorelaxation of rat or guinea pig aortic rings (similar to LxA_4) (218) Inhibits proliferation of human A549 cells, but less potently than 15-epi LxB_5 (47)

15-epi LxA_4	Inhibits leukocyte-endothelium interactions in mice (219) Blocks reactive oxygen species generation in human endothelial cells (220) Stimulates human monocyte chemotaxis (221) Inhibits proliferation of human A549 cells, but less potently than 15-epi LxB_5 (47)
15-epi LxB_4	Inhibits proliferation of human A549 cells, more potently than 15-epi LxA_4 or LxB_4 (47)
C. CYP oxylipins	
5,6-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (222) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (223)
8,9-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (222) Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers (224) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (223)
11,12-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (222) Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers (224) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (223) Relaxes porcine coronary artery with similar potency as its EpETrE isomer (225)
14,15-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (222) Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers (224) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (223) Most potent PPARα activator in a monkey COS-7 cell expression system when compared to other DiHETrE and EpETrE isomers (226) Stimulates metastasis and escape from tumor dormancy in several murine tumor models (227)
5,6-EpETrE	Vasodilatory effects in intestinal microcirculation in rat model (228) Promotes angiogenesis by stimulating endothelial cell proliferation in vitro and angiogenesis in vivo in murine model (229) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (224) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (222)
8,9-EpETrE	Promotes angiogenesis by stimulating endothelial cell proliferation in vitro and angiogenesis in vivo (229) Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine models (230) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation (231) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (224) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (222)
11,12-	Vasodilatory effects in intestinal microcirculation (228)

EpETrE	Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine models (230) Inhibits vascular inflammation distinct from its vasodilatory effects by inhibiting nuclear factor-κB and IκB kinase in murine model (232) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation (231) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (224) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (222) Relaxes porcine coronary artery with similar potency as its DiHETrE isomer (225) Enhances angiogenesis and tumor progression in murine model (233)
14,15-EpETrE	Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine model (230) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation (231) Vasodilates U46619-preconstricted bovine coronary artery rings, more potently than 14,15-DiHETrE (234) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (224) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (222) Antinociceptive effect in thermally produced tail-flick response in rats, while other regioisomers were not effective at same dose (235) Enhances angiogenesis and tumor progression (233)
16-HETE	Induces vasodilation in isolated rabbit kidney (236) Inhibits human leukocyte activation (237) Decreases intracranial pressure in a rabbit model of stroke (237)
17-HETE	Inhibits rabbit proximal tubule ATPase activity, but has no renal vasodilatory activity (236)
18-HETE	Induces vasodilation in isolated rabbit kidney (236)
19-HETE	Reduces pressure in rabbit-perfused kidneys (238) Induces vasodilation in canine renal arteries (239) Stimulates rat renal Na^+/K^+-ATPase (240)
20-HETE	Reduces pressure in rabbit-perfused kidneys (238) Induces vasoconstriction in canine renal arteries (239) and porcine coronary arteries (241) Stimulates inflammatory cytokine production in human endothelial cells (242) Stimulates proliferation of rat vascular smooth muscle cells (243)

Table 2. Examples of LA derived oxylipin functions

A. LOX oxylipins	
9-HODE	Induces endoplasmic reticulum stress in human macrophages (244) Inhibits proliferation and induces apoptosis in human U937 cells (245) Pro-inflammatory in skin under oxidative conditions in human (246) Induces maturation, scavenger receptor expression and activates PPARγ-dependent transcription in human monocytes (247) Does not inhibit tumor cell adhesion to endothelial cells (cf. 13-HODE) in mice (178)
9-oxo-ODE	Activates PPARγ-dependent transcription in human monocytes (as does 9-HODE and -HpODE) (247)
13-HODE	Prevents platelets from adhering to human vascular endothelium (248) Decreases thrombin-induced platelet adherence to other platelets and to the endothelial cells *in vitro (249)* Induces maturation, scavenger receptor expression and activates PPARγ-dependent transcription in human monocytes (247) *Inhibits proliferation of* hyperproliferative skin in guinea pigs (250) Inhibits tumor cell adhesion to endothelial cells (178) Inhibits the secretion and assembly of triacylglycerol-rich lipoprotein particles in vitro (251) Inhibits human neutrophil production of LtB_4 in vitro (72)
13-HpODE	Relaxes canine circumflex and splenic arteries, similarly to 13-HODE (252) Relaxes human pulmonary arteries (186)
13-oxo-ODE	Reduces inflammation in human colonic epithelial cells (253) Does not inhibit tumor cell adhesion to endothelial cells (cf. 13-HODE) in mice (178) Does not inhibit LOX enzyme activity (cf. 12- and 15-oxo-ETE) in vitro (171) Activates PPARγ-dependent transcription in human monocytes (as does 13-HODE and -HpODE) (247)
B. CYP oxylipins	
9,10-DiHOME	Decreases left ventricular developed pressure recovery and increases coronary resistance following ischemia/reperfusion in mouse heart (254) Causes mitochondrial dysfunction, leading to cell death in rabbit renal proximal tubular cells, while parent epoxy compound is not toxic (255)
12,13-DiHOME	Causes mitochondrial dysfunction, leading to cell death in rabbit renal proximal tubular cells, while parent epoxy compound is not toxic (255) Causes acute respiratory distress syndrome in mice; more toxic than its epoxy parent (256) Lacks protective effect of 12,13-EpOME in rabbit renal proximal tubular cells exposed to hypoxia/reoxygenation (257)
9,10-EpOME	Inhibits mitochondrial respiration in perfused rat lung (258) Relaxes rat stomach smooth muscle and uncouples mitochondrial respiration (259) Induces canine heart failure when injected intravenously (260) Inhibits growth of normal and transformed human cells in culture (261) Induces vasoconstriction in isolated perfused cat carotid arteries (262)
12,13-EpOME	Pre-treatment with low concentrations maintains mitochondrial respiration in rabbit renal proximal tubular cells exposed to hypoxia/reoxygenation; 12,13-DiHOME has no effect (257) Induces vasoconstriction in isolated perfused cat carotid arteries (262) Induces dysfunction in isolated rabbit renal cortical mitochondria, while 12,13-DiHOME does not (263)

Table 3. Examples of DGLA derived oxylipin functions

A. COX oxylipins	
PGD_1	Activates pro-inflammatory receptor CRTH2/DP2 in human kidney cells (cf. PGE_1) (264)
	Inhibits human platelet aggregation, but is 100-fold less potent than PGD_2 or PGD_3 (121)
PGE_1	Does not activate pro-inflammatory receptor CRTH2/DP2 in human kidney cells (cf. PGD_1)
	Reduces healing time of lower limb ulcers in human patients (265)
	Alleviates neurological deteriorations of diabetic rats (266)
	Vasodilates rat coronary and systemic circulation (267)
	Stimulates peripheral blood flow in humans with peripheral arterial disease (268)
	Reduces pulmonary hypertension in patients with pulmonary arterial hypertension (269)
	Inhibits human platelet aggregation (122, 270)
13,14-dihydro-PGE_1	Inhibits human platelet aggregation with similar potency to PGE_1 (270)
B. LOX oxylipins	
12-HETrE	Enhances delayed-type hypersensitivity in guinea pig model (271)
	Inhibits human platelet aggregation (272)
15-HETrE	Inhibits epidermal hyperproliferation in guinea pig skin (69, 273)
	Inhibits formation of pro-inflammatory LtB_4 in human neutrophils (72)
	Inhibits cellular growth and AA metabolism in human prostatic adenocarcinoma cells (274)

Table 4. Examples of AdA derived oxylipin functions

A. COX oxylipins	
Dihomo-PGE_2	Stimulates cAMP production in rabbit renal medullary interstitial cells more potently than dihomo-PGI_2, but 10 times less potently than PGE_2 (79)
	No contractile activity in vascular and non-vascular smooth muscle tissue at levels that PGE_2 had significant activity (79)
Dihomo-PGI_2	Inhibits thrombin-induced human platelet aggregation, but is 100-fold less potent than PGI_2 (77)
	Stimulates cAMP production in rabbit renal medullary interstitial cells, but 100 times less potently than PGI_2 (79)
Dihomo-TxA_2	No contractile activity in rabbit aorta (79) [compared to constrictory effect of TxA_2 (145)]
B. CYP oxylipins	
Dihomo-7,8-, Dihomo-10,11-, Dihomo-13,14-, Dihomo-16,17-EpETrE	Induce vasorelaxation in bovine coronary arterial rings (81)
	Dilate canine and porcine coronary microvessels with similar potency to other dihomo-EpETE isomers as well as EpETrE and EpEPE isomers (230)
Dihomo-16,17-EpETrE	Causes concentration-related relaxations in pre-constricted bovine adrenal cortical arteries (78)

Table 5. Examples of ALA derived oxylipin functions

A. COX oxylipins	
9-HOTrE	Associated with glomerular hypertrophy in obese rats (57)
9,16-diHOTrE	Inhibits prostaglandin synthesis from COX1 and collagen induced human platelet aggregation (82)
13-HOTrE	Suppresses IL-1β induced expression of matrix metalloproteinases in human chondrocytes in vitro (275) Associated with glomerular hypertrophy in obese rats (57)
13-HpOTrE	Causes moderate and reversible depression in action potential parameters in rat cardiomyocytes (276)
13-oxo-OTrE	Induces glucose uptake and promotes adipocyte differentiation in murine model (277)
B. CYP oxylipins	
9,10-DiHODE	Lower in blood of hyperlipidemic vs. normolipidemic persons (56)
12,13-DiHODE	Lower in blood of hyperlipidemic vs. normolipidemic persons (56)

Table 6. Examples of EPA derived oxylipin functions

A. COX oxylipins	
15-deoxy-PGJ	Increases adiponectin secretion from murine adipocytes (278)
PGD_3	Lowers intraocular pressure in rabbit model (279)
	Decreases peripheral vascular resistance and increases cardiac output and heart rate in dogs (280)
	As potent as PGD_2 in modulating sympathetic nerve transmission in the eye but less effective in activating vagally mediated bradycardia in cat model (281)
	Inhibits human platelet aggregation with similar or greater activity than PGD_2 (121, 122)
PGE_3	Lowers intraocular pressure but caused mild conjunctival hyperemia in rabbit model (279)
	Compared to PGE_2, is not mitogenic to and is less efficient in inducing COX2 gene expression in murine NIH 3T3 fibroblasts, and less efficient in inducing IL-6 synthesis in murine RAW 264.7 macrophages (282)
	Inhibits proliferation of human A549 cells (283) and mouse melanoma B16 cells (284)
	Less effective than PGE_2 in elevating plasma noradrenaline when administered intracerebroventricularly in rats (285)
	Less potent stimulator of cAMP production than PGE_2 in HEK293 human renal cells (83)
$PGF_{3\alpha}$	Less protective than $PGF_{2\alpha}$ on ethanol induced gastric mucosal injury in rat model (286)
PGI_3	Inhibits aggregation in human and rabbit platelets (287, 288)
	Promotes relaxation of bovine coronary arteries (288)
Δ^{12}-PGJ_3	Inhibits progression of leukemia in a mouse model (289)
TxA_3	Synthesized at a much lower rate than TxA_2 in human platelets (288)
	Elevates catecholamines when administered intracerebroventricularly as potently as TxA_2 in rats (285)
	Activates human platelet aggregation with potency comparable with TxA_2 (83)
B. LOX oxylipins	
5-HEPE	Enhances glucose-dependent insulin secretion in mouse MIN6 insulinoma cells and human NuTu80 intestinal carcinoma cells (290)
	Promotes bovine neutrophil chemotaxis in vitro, but less potently than 5-HETE (167)
5-HpETE	Inhibits human platelet aggregation, but less effectively than 12-HpEPE (168)
5-oxo-EPE	Stimulates migration of both human neutrophils and eosinophils at one tenth the activity of 5-oxo-ETE (88)
8-HEPE	Induces adipogenesis in mouse pre-adipocytes and glucose uptake in myoblasts via PPAR activation (4)
9-HEPE	Induces adipogenesis in mouse pre-adipocytes and glucose uptake in myoblasts via PPAR activation (4)
12-HEPE	Inhibits human platelet aggregation similarly to 12-HETE, but less effectively than 12-HPEPE or 12-HPETE (168)
12-HpEPE	Inhibits human platelet aggregation similarly to 12-HpETE, and more potently than 5- or 15-HpEPE (168, 184)
15-HEPE	Inhibits 5-LOX in rat basophilic leukemia cells (84)
	Inhibits cellular growth and AA metabolism in human prostatic adenocarcinoma cells (274)
15-HpEPE	Inhibits human platelet aggregation similarly to 15-HpETE, but less potently than 12-HpEPE (168)
	Inhibits glucosamine synthetase activity in rabbit gastric mucosa (291)
	Decreases rabbit renal prostaglandin synthesis (292)
	Inhibits AA metabolism in rabbit platelets (293)
LtA_5	Inhibits the formation of LtB_4 from LtA_4 by rat and human neutrophil LtA_4 hydrolase

	(294)
LtB_5	Less active than LtB_4 in aggregating rat and human neutrophils (85) Promotes chemotaxis of bovine or human neutrophils, but is much less potent than LtB_4 (167, 207)
LtC_5	Contracts guinea pig lung parenchymal strips and ileal tissues, with similar potency to LtC_4 (207) Inhibits the anaphylactic reaction in guinea pig isolated heart, with similar potency as LtC_4 (295) Contracts guinea pig ileum but less potently than LtC_4 (296)
LtD_5	Inhibited IL-1β-induced COX2 expression in human pulmonary microvascular endothelial cells (297) Stimulates volume regulation in murine Ehrlich ascites tumor cells (similar potency as LtD_4) (298)
LxA_5	Induces contraction of isolated guinea pig pulmonary smooth muscle (similar to LxA_4 and LxB_4 effects), but does not induce vasorelaxation of rat or guinea pig aortic rings (unlike LxA_4 and LxB_4) (218) Induces superoxide anion generation from canine neutrophils and contraction of rat tail arteries (299)
LxB_5	Does not induce contraction of isolated guinea pig pulmonary smooth muscle (unlike LxA_5, LxA_4 and LxB_4) or vasorelaxation of rat or guinea pig aortic rings (unlike LxA_4 and LxB_4) (218) Induces superoxide anion generation from canine neutrophils (with similar activity to 4-series Lx) (299)
C. CYP oxylipins	
8,9-, 11,12-, 14,15-, 17,18-DiHETE	Inhibit human platelet aggregation, but with much less potency than parent EpETE (300)
8,9-, 11,12-, 14,15-, 17,18-EpETE	Dilate canine and porcine coronary microvessels with similar potency to other EpETE isomers as well as EpETrE and dihomo-EpETrE isomers (230)
	Inhibit human platelet aggregation and thromboxane synthesis, with similar potency to other EpETE and EDPE isomers, and greater potency than EpETrE isomers (300)
17,18-EpETE	Decreases human platelet aggregation (301) Relaxing effect on human bronchi arterial and airway smooth muscles (302) Anti-inflammatory effect in human lungs (303) Vasodilator in rat vascular smooth muscle cells (304)
18-HEPE	Inhibits macrophage mediated inflammation in cardiac fibroblasts in culture and prevents pressure overload–induced cardiac fibrosis and inflammation in mice (305) Decreases lipopolysaccharide-induced TNFα secretion in murine macrophage cell line (306)
RvE1	Reduces dermal inflammation, peritonitis, dendritic cell migration, and IL-12 production in an inflammatory mouse model (307) Reduces total leukocytes and PMN infiltration in murine peritonitis (308) Reduces hepatic fibrosis in murine model of infection (309) Promotes phagocyte removal during acute inflammation *in vitro* and *in vivo* (310)
RvE2	Stops zymogen-induced PMN leukocyte infiltration in murine peritonitis (311) Enhances phagocytosis and anti-inflammatory cytokine production in murine peritonitis (312) Inhibits human neutrophil infiltration and proinflammatory cytokines in an acute peritonitis (313)
RvE3	Inhibits neutrophil chemotaxis in vitro and reduces neutrophil numbers in zymosan-induced murine peritonitis in vivo (91) Blocks PMN infiltration in mouse model of peritonitis (314)

Table 7. Examples of DHA derived oxylipin functions

A. LOX oxylipins	
14,20-DiHDoHE	Inhibits PMN infiltration in the mouse peritonitis model (103)
14,21-DiHDoHE	Enhances wound healing in murine models (315, 316)
4-HDoHE	Inhibits endothelial cell proliferation and sprouting angiogenesis in mouse model of oxygen-induced retinopathy (317)
7-HDoHE	Activates PPARγ in transfected monkey kidney COS-7 cells (318)
13-HDoHE	Inhibits TNFα induced cytokine production in human microglial cells (28)
14-HDoHE	Inhibits human platelet aggregation (182)
17*S*-HDoHE	Vasodilates bovine coronary arterial smooth muscle cells (319)
	Reduces genotoxic and oxidative damage in murine hepatocyte cells and TNFα release by murine macrophages (318)
17*R*-HDoHE	Inhibits hyperalgesia in a rat model of adjuvant-induced arthritis (320)
	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (321)
	Inhibits TNFα induced cytokine production in human microglial cells (28)
17-HDoHE	Decreases lipopolysaccharide-induced TNFα secretion in murine macrophage cell line (306)
	Inhibits 5-LOX in rat basophilic leukemia cells (84)
17-HpDoHE	Displays cytotoxic potency in human neuroblastoma cells (322)
MaR1	Anti-inflammatory in a murine model of acute respiratory distress syndrome (323)
	Reduces inflammation- and chemotherapy-induced neuropathic pain in mice (324)
	Mitigates inflammatory effects of lipopolysaccharide-induced lung injury in mouse model (325)
PD1	Reduces genotoxic and oxidative damage in murine hepatocyte cells and TNFα release by murine macrophages (318)
	Promotes murine phagocyte removal during acute inflammation *in vitro* and *in vivo* (310)
	Decreases leukocyte accumulation in a mouse model of kidney injury (326)
	Protects human retinal pigment epithelial cells from apoptosis due to oxidative stress (327)
	Promotes mouse corneal epithelial cell wound healing (214)
PDX	Reduces inflammation in murine peritonitis and inhibits human microglial cell cytokine expression in vitro (93)
	Inhibits collagen, AA, and thromboxane induced human platelet aggregation (328)
	Inhibits PMN infiltration in mouse model of ischemic stroke (329)
	Decreases reactive oxygen species production and COX activity in human neutrophils (330)
	Improves insulin sensitivity by raising muscle IL-6 without affecting adipose tissue inflammation in murine model (331)
RvD1	Reduces reactivity and Ca^{2+} sensitivity in overactive human pulmonary artery smooth muscle cells (332)
	Improves bacterial clearance and survival of mice with cecal ligation and puncture induced sepsis (333)
RvD2	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (321)
	Improves bacterial clearance and survival of mice with cecal ligation and puncture induced sepsis (334)
	Inhibits inflammatory pain in mice (335)
	Mitigates neutrophil-mediated damage in mouse burn model (336)
RvD3	Reduces peritonitis and dermal inflammation in murine model (337)
RvD5	Enhances phagocyte containment of Escherichia coli in a mouse model (338)
AT-RvD1	Inhibits hyperalgesia in a rat model of adjuvant-induced arthritis (320)

	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (321)
AT-RvD3	Reduces murine peritonitis and dermal inflammation with activity similar to RvD3 (337)
B. CYP oxylipins	
7,8-, 10,11-, 13,14-, 16,17-, 19,20-DiHDPE	Inhibit human platelet aggregation with moderately lower potency to EpDPE, and do not affect thromboxane synthesis (300)
13,14-, 16,17-DiHDPE	Reduce pain associated with inflammation more potently than EpETrE and EpEPE (93)
13,14-DiHDPE	Markedly reduces potency to dilate porcine coronary arterioles compared to parent compound (339)
7,8-, 10,11-, 13,14-, 16,17-, 19,10-EpDPE	Dilates porcine coronary arterioles (339) Inhibits human platelet aggregation and thromboxane synthesis, with similar potency to other EpETE and EpDPE isomers, and greater potency than EpETrE isomers (300)
16,17-, 19,20-EpDPE	Inhibits Met-1 tumor angiogenesis and growth in mice (233)
19,20-EpDPE	Decreases human platelet aggregation (301)

Literature Cited

1. Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH, Jr., Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, et al. A comprehensive classification system for lipids. Journal of lipid research 2005;46(5):839-61. doi: 10.1194/jlr.E400004-JLR200.
2. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA. Update of the LIPID MAPS comprehensive classification system for lipids. Journal of lipid research 2009;50 Suppl:S9-14. doi: 10.1194/jlr.R800095-JLR200.
3. Balvers MG, Verhoeckx KC, Bijlsma S, Rubingh CM, Meijerink J, Wortelboer HM, Witkamp RF. Fish oil and inflammatory status alter the n-3 to n-6 balance of the endocannabinoid and oxylipin metabolomes in mouse plasma and tissues. Metabolomics : Official journal of the Metabolomic Society 2012;8(6):1130-47. doi: 10.1007/s11306-012-0421-9.
4. Yamada H, Oshiro E, Kikuchi S, Hakozaki M, Takahashi H, Kimura K. Hydroxyeicosapentaenoic acids from the Pacific krill show high ligand activities for PPARs. Journal of lipid research 2014;55(5):895-904. doi: 10.1194/jlr.M047514.
5. Schebb NH, Ostermann AI, Yang J, Hammock BD, Hahn A, Schuchardt JP. Comparison of the effects of long-chain omega-3 fatty acid supplementation on plasma levels of free and esterified oxylipins. Prostaglandins & other lipid mediators 2014;113-115:21-9. doi: 10.1016/j.prostaglandins.2014.05.002.
6. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chemical reviews 2011;111(10):6130-85. doi: 10.1021/cr200085w.

7. Reed KA, Tucker DE, Aloulou A, Adler D, Ghomashchi F, Gelb MH, Leslie CC, Oates JA, Boutaud O. Functional characterization of mutations in inherited human cPLA(2) deficiency. Biochemistry 2011;50(10):1731-8. doi: 10.1021/bi101877n.
8. Adler DH, Cogan JD, Phillips JA, 3rd, Schnetz-Boutaud N, Milne GL, Iverson T, Stein JA, Brenner DA, Morrow JD, Boutaud O, et al. Inherited human cPLA(2alpha) deficiency is associated with impaired eicosanoid biosynthesis, small intestinal ulceration, and platelet dysfunction. The Journal of clinical investigation 2008;118(6):2121-31. doi: 10.1172/JCI30473.
9. Dichlberger A, Schlager S, Maaninka K, Schneider WJ, Kovanen PT. Adipose triglyceride lipase regulates eicosanoid production in activated human mast cells. Journal of lipid research 2014;55(12):2471-8. doi: 10.1194/jlr.M048553.
10. Schewe T, Halangk W, Hiebsch C, Rapoport SM. A lipoxygenase in rabbit reticulocytes which attacks phospholipids and intact mitochondria. FEBS Lett 1975;60(1):149-52.
11. Belkner J, Wiesner R, Kuhn H, Lankin VZ. The oxygenation of cholesterol esters by the reticulocyte lipoxygenase. FEBS Lett 1991;279(1):110-4.
12. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001;294(5548):1871-5. doi: 10.1126/science.294.5548.1871.
13. Buczynski MW, Dumlao DS, Dennis EA. Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology. Journal of lipid research 2009;50(6):1015-38. doi: 10.1194/jlr.R900004-JLR200.
14. Bos CL, Richel DJ, Ritsema T, Peppelenbosch MP, Versteeg HH. Prostanoids and prostanoid receptors in signal transduction. The international journal of biochemistry & cell biology 2004;36(7):1187-205. doi: 10.1016/j.biocel.2003.08.006.
15. Spector AA, Kim HY. Cytochrome P epoxygenase pathway of polyunsaturated fatty acid metabolism. Biochim Biophys Acta 2014. doi: 10.1016/j.bbalip.2014.07.020.
16. Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 2014;40(3):315-27. doi: 10.1016/j.immuni.2014.02.009.
17. Kuhn H, Banthiya S, van Leyen K. Mammalian lipoxygenases and their biological relevance. Biochimica et biophysica acta 2014. doi: 10.1016/j.bbalip.2014.10.002.
18. Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochimica et biophysica acta 2014. doi: 10.1016/j.bbalip.2014.08.006.
19. Shahabi P, Siest G, Meyer UA, Visvikis-Siest S. Human cytochrome P450 epoxygenases: Variability in expression and role in inflammation-related disorders. Pharmacology & therapeutics 2014;144(2):134-61. doi: 10.1016/j.pharmthera.2014.05.011.
20. Konkel A, Schunck WH. Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. Biochimica et biophysica acta 2011;1814(1):210-22. doi: 10.1016/j.bbapap.2010.09.009.
21. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science 1987;237(4819):1171-6.
22. Smith WL, Urade Y, Jakobsson PJ. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chemical reviews 2011;111(10):5821-65. doi: 10.1021/cr2002992.
23. Pace-Asciak CR. Pathophysiology of the hepoxilins. Biochimica et biophysica acta 2014. doi: 10.1016/j.bbalip.2014.09.007.

24. Fan YY, Chapkin RS. Mouse peritoneal macrophage prostaglandin E1 synthesis is altered by dietary gamma-linolenic acid. The Journal of nutrition 1992;122(8):1600-6.
25. Kulkarni PS, Srinivasan BD. Eicosapentaenoic acid metabolism in human and rabbit anterior uvea. Prostaglandins 1986;31(6):1159-64.
26. O'Neill GP, Mancini JA, Kargman S, Yergey J, Kwan MY, Falgueyret JP, Abramovitz M, Kennedy BP, Ouellet M, Cromlish W, et al. Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. Molecular pharmacology 1994;45(2):245-54.
27. Thuresson ED, Lakkides KM, Smith WL. Different catalytically competent arrangements of arachidonic acid within the cyclooxygenase active site of prostaglandin endoperoxide H synthase-1 lead to the formation of different oxygenated products. The Journal of biological chemistry 2000;275(12):8501-7.
28. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. The Journal of experimental medicine 2002;196(8):1025-37.
29. Laneuville O, Breuer DK, Xu N, Huang ZH, Gage DA, Watson JT, Lagarde M, DeWitt DL, Smith WL. Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2. Formation of 12-hydroxy-(9Z, 13E/Z, 15Z)- octadecatrienoic acids from alpha-linolenic acid. J Biol Chem 1995;270(33):19330-6.
30. Feltenmark S, Gautam N, Brunnstrom A, Griffiths W, Backman L, Edenius C, Lindbom L, Bjorkholm M, Claesson HE. Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells. Proceedings of the National Academy of Sciences of the United States of America 2008;105(2):680-5. doi: 10.1073/pnas.0710127105.
31. Underwood KW, Song C, Kriz RW, Chang XJ, Knopf JL, Lin LL. A novel calcium-independent phospholipase A2, cPLA2-gamma, that is prenylated and contains homology to cPLA2. J Biol Chem 1998;273(34):21926-32.
32. Grandits M, Oostenbrink C. Selectivity of cytosolic phospholipase A2 type IV toward arachidonyl phospholipids. J Mol Recognit 2015. doi: 10.1002/jmr.2462.
33. Buczynski MW, Dumlao DS, Dennis EA. An integrated omics analysis of eicosanoid biology (vol 50, pg 1015, 2009). Journal of lipid research 2009;50(7):1505-. doi: DOI 10.1194/jlr.R900004ERR-JLR200.
34. Sandig H, Pease JE, Sabroe I. Contrary prostaglandins: the opposing roles of PGD(2) and its metabolites in leukocyte function. J Leukocyte Biol 2007;81(2):372-82. doi: Doi 10.1189/Jlb.0706424.
35. Catella F, Healy D, Lawson JA, Fitzgerald GA. 11-Dehydrothromboxane-B2 - a Quantitative Index of Thromboxane-A2 Formation in the Human Circulation. Proceedings of the National Academy of Sciences of the United States of America 1986;83(16):5861-5. doi: DOI 10.1073/pnas.83.16.5861.
36. Sutherland M, Shankaranarayanan P, Schewe T, Nigam S. Evidence for the presence of phospholipid hydroperoxide glutathione peroxidase in human platelets: implications for its involvement in the regulatory network of the 12-lipoxygenase pathway of arachidonic acid metabolism. Biochem J 2001;353(Pt 1):91-100.

37. Goetzl EJ, Sun FF. Generation of unique mono-hydroxy-eicosatetraenoic acids from arachidonic acid by human neutrophils. The Journal of experimental medicine 1979;150(2):406-11.
38. Yamada M, Proia AD. 8(S)-hydroxyeicosatetraenoic acid is the lipoxygenase metabolite of arachidonic acid that regulates epithelial cell migration in the rat cornea. Cornea 2000;19(3 Suppl):S13-20.
39. Fruteau de Laclos B, Maclouf J, Poubelle P, Borgeat P. Conversion of arachidonic acid into 12-oxo derivatives in human platelets. A pathway possibly involving the heme-catalysed transformation of 12-hydroperoxy-eicosatetraenoic acid. Prostaglandins 1987;33(3):315-37.
40. Erlemann KR, Cossette C, Gravel S, Lesimple A, Lee GJ, Saha G, Rokach J, Powell WS. Airway epithelial cells synthesize the lipid mediator 5-oxo-ETE in response to oxidative stress. Free radical biology & medicine 2007;42(5):654-64. doi: 10.1016/j.freeradbiomed.2006.12.006.
41. O'Flaherty JT, Wykle RL, Redman J, Samuel M, Thomas M. Metabolism of 5-hydroxyicosatetraenoate by human neutrophils: production of a novel omega-oxidized derivative. Journal of immunology 1986;137(10):3277-83.
42. Tejera N, Boeglin WE, Suzuki T, Schneider C. COX-2-dependent and -independent biosynthesis of dihydroxy-arachidonic acids in activated human leukocytes. J Lipid Res 2012;53(1):87-94. doi: 10.1194/jlr.M017822.
43. Bryant RW, Bailey JM. Altered lipoxygenase metabolism and decreased glutathione peroxidase activity in platelets from selenium-deficient rats. Biochemical and biophysical research communications 1980;92(1):268-76.
44. Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proceedings of the National Academy of Sciences of the United States of America 1984;81(17):5335-9.
45. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: An update. Biochim Biophys Acta 2010;1801(12):1260-73. doi: 10.1016/j.bbalip.2010.08.002.
46. Romano M, Chen XS, Takahashi Y, Yamamoto S, Funk CD, Serhan CN. Lipoxin synthase activity of human platelet 12-lipoxygenase. The Biochemical journal 1993;296 (Pt 1):127-33.
47. Claria J, Lee MH, Serhan CN. Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. Molecular medicine 1996;2(5):583-96.
48. Titos E, Chiang N, Serhan CN, Romano M, Gaya J, Pueyo G, Claria J. Hepatocytes are a rich source of novel aspirin-triggered 15-epi-lipoxin A(4). The American journal of physiology 1999;277(5 Pt 1):C870-7.
49. Birnbaum Y, Ye Y, Lin Y, Freeberg SY, Huang MH, Perez-Polo JR, Uretsky BF. Aspirin augments 15-epi-lipoxin A4 production by lipopolysaccharide, but blocks the pioglitazone and atorvastatin induction of 15-epi-lipoxin A4 in the rat heart. Prostaglandins & other lipid mediators 2007;83(1-2):89-98. doi: 10.1016/j.prostaglandins.2006.10.003.
50. Guido DM, McKenna R, Mathews WR. Quantitation of hydroperoxy-eicosatetraenoic acids and hydroxy-eicosatetraenoic acids as indicators of lipid peroxidation using gas

chromatography-mass spectrometry. Analytical biochemistry 1993;209(1):123-9. doi: 10.1006/abio.1993.1091.
51. Musiek ES, Yin H, Milne GL, Morrow JD. Recent advances in the biochemistry and clinical relevance of the isoprostane pathway. Lipids 2005;40(10):987-94.
52. Oliw EH, Bylund J, Herman C. Bisallylic hydroxylation and epoxidation of polyunsaturated fatty acids by cytochrome P450. Lipids 1996;31(10):1003-21.
53. Bylund J, Kunz T, Valmsen K, Oliw EH. Cytochromes P450 with bisallylic hydroxylation activity on arachidonic and linoleic acids studied with human recombinant enzymes and with human and rat liver microsomes. The Journal of pharmacology and experimental therapeutics 1998;284(1):51-60.
54. Bylund J, Ericsson J, Oliw EH. Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography-mass spectrometry with ion trap MS. Analytical biochemistry 1998;265(1):55-68.
55. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, et al. The human serum metabolome. PloS one 2011;6(2):e16957. doi: 10.1371/journal.pone.0016957.
56. Schuchardt JP, Schmidt S, Kressel G, Dong H, Willenberg I, Hammock BD, Hahn A, Schebb NH. Comparison of free serum oxylipin concentrations in hyper- vs. normolipidemic men. Prostaglandins, leukotrienes, and essential fatty acids 2013;89(1):19-29. doi: 10.1016/j.plefa.2013.04.001.
57. Caligiuri SP, Love K, Winter T, Gauthier J, Taylor CG, Blydt-Hansen T, Zahradka P, Aukema HM. Dietary linoleic acid and alpha-linolenic acid differentially affect renal oxylipins and phospholipid fatty acids in diet-induced obese rats. The Journal of nutrition 2013;143(9):1421-31. doi: 10.3945/jn.113.177360.
58. Reinaud O, Delaforge M, Boucher JL, Rocchiccioli F, Mansuy D. Oxidative metabolism of linoleic acid by human leukocytes. Biochemical and biophysical research communications 1989;161(2):883-91.
59. Bull AW, Earles SM, Bronstein JC. Metabolism of oxidized linoleic acid: distribution of activity for the enzymatic oxidation of 13-hydroxyoctadecadienoic acid to 13-oxooctadecadienoic acid in rat tissues. Prostaglandins 1991;41(1):43-50.
60. Askari AA, Thomson S, Edin ML, Lih FB, Zeldin DC, Bishop-Bailey D. Basal and inducible anti-inflammatory epoxygenase activity in endothelial cells. Biochemical and biophysical research communications 2014;446(2):633-7. doi: 10.1016/j.bbrc.2014.03.020.
61. Larsson N, Lundstrom SL, Pinto R, Rankin G, Karimpour M, Blomberg A, Sandstrom T, Pourazar J, Trygg J, Behndig AF, et al. Lipid mediator profiles differ between lung compartments in asthmatic and healthy humans. The European respiratory journal 2014;43(2):453-63. doi: 10.1183/09031936.00209412.
62. Niki E, Yoshida Y. Biomarkers for oxidative stress: measurement, validation, and application. The journal of medical investigation : JMI 2005;52 Suppl:228-30.
63. Funk CD, Powell WS. Metabolism of linoleic acid by prostaglandin endoperoxide synthase from adult and fetal blood vessels. Biochimica et biophysica acta 1983;754(1):57-71.
64. Hamberg M. Omega 6-oxygenation of 6, 9, 12-octadecatrienoic acid in human platelets. Biochemical and biophysical research communications 1983;117(2):593-600.

65. Laethem RM, Balazy M, Koop DR. Epoxidation of C18 unsaturated fatty acids by cytochromes P4502C2 and P4502CAA. Drug metabolism and disposition: the biological fate of chemicals 1996;24(6):664-8.
66. Directory Patent. Available from: http://www.directorypatent.com/U2S/20070248586-a1.html (accessed January 13 2015).
67. Amagai Y, Oida K, Matsuda A, Jung K, Kakutani S, Tanaka T, Matsuda K, Jang H, Ahn G, Xia Y, et al. Dihomo-gamma-linolenic acid prevents the development of atopic dermatitis through prostaglandin D1 production in NC/Tnd mice. J Dermatol Sci 2015;79(1):30-7. doi: 10.1016/j.jdermsci.2015.03.010.
68. Manku MS, Oka M, Horrobin DF. Differential regulation of the formation of prostaglandins and related substances from arachidonic acid and from dihomogammalinolenic acid. II. Effects of vitamin C. Prostaglandins Med 1979;3(2):129-37.
69. Xi S, Pham H, Ziboh WA. 15-hydroxyeicosatrienoic acid (15-HETrE) suppresses epidermal hyperproliferation via the modulation of nuclear transcription factor (AP-1) and apoptosis. Archives of dermatological research 2000;292(8):397-403.
70. Miller CC, Ziboh VA. Gammalinolenic acid-enriched diet alters cutaneous eicosanoids. Biochemical and biophysical research communications 1988;154(3):967-74.
71. Miller CC, McCreedy CA, Jones AD, Ziboh VA. Oxidative metabolism of dihomogammalinolenic acid by guinea pig epidermis: evidence of generation of anti-inflammatory products. Prostaglandins 1988;35(6):917-38.
72. Iversen L, Fogh K, Bojesen G, Kragballe K. Linoleic acid and dihomogammalinolenic acid inhibit leukotriene B4 formation and stimulate the formation of their 15-lipoxygenase products by human neutrophils in vitro. Evidence of formation of antiinflammatory compounds. Agents and actions 1991;33(3-4):286-91.
73. Heitmann J, Iversen L, Kragballe K, Ziboh VA. Incorporation of 15-hydroxyeicosatrienoic acid in specific phospholipids of cultured human keratinocytes and psoriatic plaques. Experimental dermatology 1995;4(2):74-8.
74. Chapkin RS, Miller CC, Somers SD, Erickson KL. Ability of 15-hydroxyeicosatrienoic acid (15-OH-20:3) to modulate macrophage arachidonic acid metabolism. Biochemical and biophysical research communications 1988;153(2):799-804.
75. Yamane M, Abe A, Yamane S. High-performance liquid chromatography-thermospray mass spectrometry of epoxy polyunsaturated fatty acids and epoxyhydroxy polyunsaturated fatty acids from an incubation mixture of rat tissue homogenate. Journal of chromatography 1994;652(2):123-36.
76. Cagen LM, Zusman RM, Pisano JJ. Formation of 1a, 1b dihomoprostaglandin E2 by rabbit renal intersititial cell cultures. Prostaglandins 1979;18(4):617-21.
77. Campbell WB, Falck JR, Okita JR, Johnson AR, Callahan KS. Synthesis of dihomoprostaglandins from adrenic acid (7,10,13,16-docosatetraenoic acid) by human endothelial cells. Biochimica et biophysica acta 1985;837(1):67-76.
78. Kopf PG, Zhang DX, Gauthier KM, Nithipatikom K, Yi XY, Falck JR, Campbell WB. Adrenic acid metabolites as endogenous endothelium-derived and zona glomerulosa-derived hyperpolarizing factors. Hypertension 2010;55(2):547-54. doi: 10.1161/HYPERTENSIONAHA.109.144147.

79. Sprecher H, VanRollins M, Sun F, Wyche A, Needleman P. Dihomo-prostaglandins and -thromboxane. A prostaglandin family from adrenic acid that may be preferentially synthesized in the kidney. The Journal of biological chemistry 1982;257(7):3912-8.
80. VanRollins M, Horrocks L, Sprecher H. Metabolism of 7,10,13,16-docosatetraenoic acid to dihomo-thromboxane, 14-hydroxy-7,10,12-nonadecatrienoic acid and hydroxy fatty acids by human platelets. Biochimica et biophysica acta 1985;833(2):272-80.
81. Yi XY, Gauthier KM, Cui L, Nithipatikom K, Falck JR, Campbell WB. Metabolism of adrenic acid to vasodilatory 1alpha,1beta-dihomo-epoxyeicosatrienoic acids by bovine coronary arteries. American journal of physiology Heart and circulatory physiology 2007;292(5):H2265-74. doi: 10.1152/ajpheart.00947.2006.
82. Liu M, Chen P, Vericel E, Lelli M, Beguin L, Lagarde M, Guichardant M. Characterization and biological effects of di-hydroxylated compounds deriving from the lipoxygenation of ALA. Journal of lipid research 2013;54(8):2083-94. doi: 10.1194/jlr.M035139.
83. Wada M, DeLong CJ, Hong YH, Rieke CJ, Song I, Sidhu RS, Yuan C, Warnock M, Schmaier AH, Yokoyama C, et al. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. J Biol Chem 2007;282(31):22254-66. doi: 10.1074/jbc.M703169200.
84. Miller C, Yamaguchi RY, Ziboh VA. Guinea pig epidermis generates putative anti-inflammatory metabolites from fish oil polyunsaturated fatty acids. Lipids 1989;24(12):998-1003.
85. Terano T, Salmon JA, Moncada S. Biosynthesis and biological activity of leukotriene B5. Prostaglandins 1984;27(2):217-32.
86. Hersberger M. Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins. Clinical chemistry and laboratory medicine : CCLM / FESCC 2010;48(8):1063-73. doi: 10.1515/CCLM.2010.212.
87. von Schacky C, Marcus AJ, Safier LB, Ullman HL, Islam N, Broekman MJ, Fischer S. Platelet-neutrophil interactions. 12S,20- and 5S,12S-dihydroxyeicosapentaenoic acids: two novel neutrophil metabolites from platelet-derived 12S-hydroxyeicosapentaenoic acid. Journal of lipid research 1990;31(5):801-10.
88. Powell WS, Gravel S, Gravelle F. Formation of a 5-oxo metabolite of 5,8,11,14,17-eicosapentaenoic acid and its effects on human neutrophils and eosinophils. Journal of lipid research 1995;36(12):2590-8.
89. Arnold C, Konkel A, Fischer R, Schunck WH. Cytochrome P450-dependent metabolism of omega-6 and omega-3 long-chain polyunsaturated fatty acids. Pharmacological reports : PR 2010;62(3):536-47.
90. Westphal C, Konkel A, Schunck WH. CYP-eicosanoids--a new link between omega-3 fatty acids and cardiac disease? Prostaglandins & other lipid mediators 2011;96(1-4):99-108. doi: 10.1016/j.prostaglandins.2011.09.001.
91. Isobe Y, Arita M, Matsueda S, Iwamoto R, Fujihara T, Nakanishi H, Taguchi R, Masuda K, Sasaki K, Urabe D, et al. Identification and structure determination of novel anti-inflammatory mediator resolvin E3, 17,18-dihydroxyeicosapentaenoic acid. J Biol Chem 2012;287(13):10525-34. doi: 10.1074/jbc.M112.340612.
92. Fer M, Dreano Y, Lucas D, Corcos L, Salaun JP, Berthou F, Amet Y. Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450.

Archives of biochemistry and biophysics 2008;471(2):116-25. doi: 10.1016/j.abb.2008.01.002.
93. Morisseau C, Inceoglu B, Schmelzer K, Tsai HJ, Jinks SL, Hegedus CM, Hammock BD. Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. J Lipid Res 2010;51(12):3481-90. doi: 10.1194/jlr.M006007.
94. Hornsten L, Bylund J, Oliw EH. Dexamethasone induces bisallylic hydroxylation of polyunsaturated fatty acids by rat liver microsomes. Archives of biochemistry and biophysics 1996;332(2):261-8. doi: 10.1006/abbi.1996.0341.
95. VanRollins M, Baker RC, Sprecher HW, Murphy RC. Oxidation of docosahexaenoic acid by rat liver microsomes. The Journal of biological chemistry 1984;259(9):5776-83.
96. Deng B, Wang CW, Arnardottir HH, Li Y, Cheng CY, Dalli J, Serhan CN. Maresin biosynthesis and identification of maresin 2, a new anti-inflammatory and pro-resolving mediator from human macrophages. PloS one 2014;9(7):e102362. doi: 10.1371/journal.pone.0102362.
97. Balas L, Guichardant M, Durand T, Lagarde M. Confusion between protectin D1 (PD1) and its isomer protectin DX (PDX). An overview on the dihydroxy-docosatrienes described to date. Biochimie 2014;99:1-7. doi: 10.1016/j.biochi.2013.11.006.
98. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510(7503):92-101. doi: 10.1038/nature13479.
99. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem 2003;278(17):14677-87. doi: 10.1074/jbc.M300218200.
100. Shinohara M, Mirakaj V, Serhan CN. Functional Metabolomics Reveals Novel Active Products in the DHA Metabolome. Frontiers in immunology 2012;3:81. doi: 10.3389/fimmu.2012.00081.
101. VanRollins M, Murphy RC. Autooxidation of docosahexaenoic acid: analysis of ten isomers of hydroxydocosahexaenoate. Journal of lipid research 1984;25(5):507-17.
102. Reynaud D, Thickitt CP, Pace-Asciak CR. Facile preparation and structural determination of monohydroxy derivatives of docosahexaenoic acid (HDoHE) by alpha-tocopherol-directed autoxidation. Analytical biochemistry 1993;214(1):165-70.
103. Yokokura Y, Isobe Y, Matsueda S, Iwamoto R, Goto T, Yoshioka T, Urabe D, Inoue M, Arai H, Arita M. Identification of 14,20-dihydroxy-docosahexaenoic acid as a novel anti-inflammatory metabolite. J Biochem 2014;156(6):315-21. doi: 10.1093/jb/mvu044.
104. Cipollina C, Salvatore SR, Muldoon MF, Freeman BA, Schopfer FJ. Generation and dietary modulation of anti-inflammatory electrophilic omega-3 fatty acid derivatives. PLoS One 2014;9(4):e94836. doi: 10.1371/journal.pone.0094836.
105. Thurnher M, Putz T, Gander H, Rahm A, Bartsch G, Ramoner R. The cyclopentenone prostaglandin PGA2 costimulates the maturation of human dendritic cells. Experimental hematology 2005;33(2):144-50. doi: 10.1016/j.exphem.2004.11.012.
106. Deliconstantinos G, Kopeikina L, Ramantanis G. PGE2 and PGA2 affect the allosteric properties and the activities of calmodulin-dependent guanylate cyclase and Ca2+-stimulated ATPase of Walker-256 tumour microsomal membranes. Anticancer research 1989;9(3):fluorescence polarization.

107. Bui T, Kuo C, Rotwein P, Straus DS. Prostaglandin A2 specifically represses insulin-like growth factor-I gene expression in C6 rat glioma cells. Endocrinology 1997;138(3):985-93. doi: 10.1210/endo.138.3.4980.
108. Fara JW, Barth KH, White RI, Jr., Bynum TE. Mesenteric vascular effects of prostaglandins F2 alpha and B2. Possible advantages over vasopressin in control of gastrointestinal bleeding. Radiology 1979;133(2):317-20. doi: 10.1148/133.2.317.
109. Hall DW, Jaitly KD. Structure-activity relationships in a series of 11-deoxy prostaglandins. Prostaglandins 1976;11(3):573-87.
110. Relic B, Benoit V, Franchimont N, Ribbens C, Kaiser MJ, Gillet P, Merville MP, Bours V, Malaise MG. 15-deoxy-delta12,14-prostaglandin J2 inhibits Bay 11-7085-induced sustained extracellular signal-regulated kinase phosphorylation and apoptosis in human articular chondrocytes and synovial fibroblasts. The Journal of biological chemistry 2004;279(21):22399-403. doi: 10.1074/jbc.M314118200.
111. Hammad H, de Heer HJ, Soullie T, Hoogsteden HC, Trottein F, Lambrecht BN. Prostaglandin D2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. Journal of immunology 2003;171(8):3936-40.
112. Urade Y, Hayaishi O. Prostaglandin D2 and sleep regulation. Biochimica et biophysica acta 1999;1436(3):606-15.
113. Forstermann U, Heldt R, Hertting G. Effects of intracerebroventricular administration of prostaglandin D2 on behaviour, blood pressure and body temperature as compared to prostaglandins E2 and F2 alpha. Psychopharmacology 1983;80(4):365-70.
114. Ueno R, Narumiya S, Ogorochi T, Nakayama T, Ishikawa Y, Hayaishi O. Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. Proceedings of the National Academy of Sciences of the United States of America 1982;79(19):6093-7.
115. Kikuchi Y, Miyauchi M, Oomori K, Kita T, Kizawa I, Kato K. Inhibition of human ovarian cancer cell growth in vitro and in nude mice by prostaglandin D2. Cancer research 1986;46(7):3364-6.
116. Sandig H, Pease JE, Sabroe I. Contrary prostaglandins: the opposing roles of PGD2 and its metabolites in leukocyte function. Journal of leukocyte biology 2007;81(2):372-82. doi: 10.1189/jlb.0706424.
117. Darius H, Michael-Hepp J, Thierauch KH, Fisch A. Inhibition of human platelets and polymorphonuclear neutrophils by the potent and metabolically stable prostaglandin D2 analog ZK 118.182. European journal of pharmacology 1994;258(3):207-13.
118. Ney P, Schror K. PGD2 and its mimetic ZK 110.841 are potent inhibitors of receptor-mediated activation of human neutrophils. Eicosanoids 1991;4(1):21-8.
119. Ward C, Dransfield I, Murray J, Farrow SN, Haslett C, Rossi AG. Prostaglandin D2 and its metabolites induce caspase-dependent granulocyte apoptosis that is mediated via inhibition of I kappa B alpha degradation using a peroxisome proliferator-activated receptor-gamma-independent mechanism. Journal of immunology 2002;168(12):6232-43.
120. Heinemann A, Schuligoi R, Sabroe I, Hartnell A, Peskar BA. Delta 12-prostaglandin J2, a plasma metabolite of prostaglandin D2, causes eosinophil mobilization from the bone marrow and primes eosinophils for chemotaxis. Journal of immunology 2003;170(9):4752-8.

121. Bundy GL, Morton DR, Peterson DC, Nishizawa EE, Miller WL. Synthesis and platelet aggregation inhibiting activity of prostaglandin D analogues. Journal of medicinal chemistry 1983;26(6):790-9.
122. Whitaker MO, Wyche A, Fitzpatrick F, Sprecher H, Needleman P. Triene prostaglandins: prostaglandin D3 and icosapentaenoic acid as potential antithrombotic substances. Proceedings of the National Academy of Sciences of the United States of America 1979;76(11):5919-23.
123. Ellis EF, Wei EP, Kontos HA. Vasodilation of cat cerebral arterioles by prostaglandins D2, E2, G2, and I2. The American journal of physiology 1979;237(3):H381-5.
124. Gray SJ, Heptinstall S. Interactions between prostaglandin E2 and inhibitors of platelet aggregation which act through cyclic AMP. European journal of pharmacology 1991;194(1):63-70.
125. Dufour M, Faes S, Dormond-Meuwly A, Demartines N, Dormond O. PGE2-induced colon cancer growth is mediated by mTORC1. Biochemical and biophysical research communications 2014;451(4):587-91. doi: 10.1016/j.bbrc.2014.08.032.
126. Harizi H, Juzan M, Pitard V, Moreau JF, Gualde N. Cyclooxygenase-2-issued prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. J Immunol 2002;168(5):2255-63.
127. Lee IT, Lin CC, Lin WN, Wu WL, Hsiao LD, Yang CM. Lung inflammation caused by adenosine-5'-triphosphate is mediated via Ca2+/PKCs-dependent COX-2/PGE2 induction. The international journal of biochemistry & cell biology 2013;45(8):1657-68. doi: 10.1016/j.biocel.2013.05.006.
128. Chou WL, Chuang LM, Chou CC, Wang AH, Lawson JA, FitzGerald GA, Chang ZF. Identification of a novel prostaglandin reductase reveals the involvement of prostaglandin E2 catabolism in regulation of peroxisome proliferator-activated receptor gamma activation. The Journal of biological chemistry 2007;282(25):18162-72. doi: 10.1074/jbc.M702289200.
129. Kelton JG, Blajchman MA. Prostaglandin I2 (prostacyclin). Canadian Medical Association journal 1980;122(2):175-9.
130. Brash AR, Jackson EK, Saggese CA, Lawson JA, Oates JA, FitzGerald GA. Metabolic disposition of prostacyclin in humans. The Journal of pharmacology and experimental therapeutics 1983;226(1):78-87.
131. Sandig H, Andrew D, Barnes AA, Sabroe I, Pease J. 9alpha,11beta-PGF2 and its stereoisomer PGF2alpha are novel agonists of the chemoattractant receptor, CRTH2. FEBS letters 2006;580(2):373-9. doi: 10.1016/j.febslet.2005.11.052.
132. Takayama K, Yuhki K, Ono K, Fujino T, Hara A, Yamada T, Kuriyama S, Karibe H, Okada Y, Takahata O, et al. Thromboxane A2 and prostaglandin F2alpha mediate inflammatory tachycardia. Nature medicine 2005;11(5):562-6. doi: 10.1038/nm1231.
133. Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T, Oida H, Yoshida N, Tanaka T, Katsuyama M, et al. Failure of parturition in mice lacking the prostaglandin F receptor. Science 1997;277(5326):681-3.
134. Morrow JD, Minton TA, Roberts LJ, 2nd. The F2-isoprostane, 8-epi-prostaglandin F2 alpha, a potent agonist of the vascular thromboxane/endoperoxide receptor, is a platelet thromboxane/endoperoxide receptor antagonist. Prostaglandins 1992;44(2):155-63.

135. Basu S. Novel cyclooxygenase-catalyzed bioactive prostaglandin F2alpha from physiology to new principles in inflammation. Medicinal research reviews 2007;27(4):435-68. doi: 10.1002/med.20098.
136. Higgs EA, Higgs GA, Moncada S, Vane JR. Prostacyclin (PGI2) inhibits the formation of platelet thrombi in arterioles and venules of the hamster cheek pouch. 1977. British journal of pharmacology 1997;120(4 Suppl):439-43; discussion 7-8.
137. Dusting GJ, Chapple DJ, Hughes R, Moncada S, Vane JR. Prostacyclin (PGI2) induces coronary vasodilatation in anaesthetised dogs. Cardiovascular research 1978;12(12):720-30.
138. Konya V, Sturm EM, Schratl P, Beubler E, Marsche G, Schuligoi R, Lippe IT, Peskar BA, Heinemann A. Endothelium-derived prostaglandin I(2) controls the migration of eosinophils. The Journal of allergy and clinical immunology 2010;125(5):1105-13. doi: 10.1016/j.jaci.2009.12.002.
139. Setty BN, Dampier CD, Stuart MJ. Arachidonic acid metabolites are involved in mediating red blood cell adherence to endothelium. The Journal of laboratory and clinical medicine 1995;125(5):608-17.
140. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature 2000;403(6765):103-8. doi: 10.1038/47520.
141. Cippitelli M, Fionda C, Di Bona D, Lupo A, Piccoli M, Frati L, Santoni A. The cyclopentenone-type prostaglandin 15-deoxy-delta 12,14-prostaglandin J2 inhibits CD95 ligand gene expression in T lymphocytes: interference with promoter activation via peroxisome proliferator-activated receptor-gamma-independent mechanisms. Journal of immunology 2003;170(9):4578-92.
142. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proceedings of the National Academy of Sciences of the United States of America 1975;72(8):2994-8.
143. Morinelli TA, Zhang LM, Newman WH, Meier KE. Thromboxane A2/prostaglandin H2-stimulated mitogenesis of coronary artery smooth muscle cells involves activation of mitogen-activated protein kinase and S6 kinase. The Journal of biological chemistry 1994;269(8):5693-8.
144. Geoffroy J, Benzoni D, Sassard J. Antihypertensive effect of thromboxane A2 receptor blockade in genetically hypertensive rats of the Lyon strain. Journal of hypertension Supplement : official journal of the International Society of Hypertension 1989;7(6):S272-3.
145. Uchida M, Iida H, Iida M, Dohi S. Changes in cerebral microcirculation during and after abdominal aortic cross-clamping in rabbits: the role of thromboxane A2 receptor. Anesth Analg 2003;96(3):651-6, table of contents.
146. Wasserman MA, Griffin RL. Thromboxane B2--comparative bronchoactivity in experimental systems. European journal of pharmacology 1977;46(4):303-13.
147. Friedman LS, Fitzpatrick TM, Bloom MF, Ramwell PW, Rose JC, Kot PA. Cardiovascular and pulmonary effects of thromboxane B2 in the dog. Circulation research 1979;44(6):748-51.
148. Kitchen EA, Boot JR, Dawson W. Chemotactic activity of thromboxane B2, prostaglandins and their metabolites for polymorphonuclear leucocytes. Prostaglandins 1978;16(2):239-44.

149. Benigni A, Chiabrando C, Perico N, Fanelli R, Patrono C, FitzGerald GA, Remuzzi G. Renal metabolism and urinary excretion of thromboxane B2 in the rat. The American journal of physiology 1989;257(1 Pt 2):F77-85.
150. Chiabrando C, Corada M, Bachi A, Fanelli R. Urinary excretion of 2,3-dinor-thromboxane B1, a major metabolite of thromboxane B2 in the rat. Prostaglandins 1994;47(6):409-22.
151. Foegh ML, Zhao Y, Madren L, Rolnick M, Stair TO, Huang KS, Ramwell PW. Urinary thromboxane A2 metabolites in patients presenting in the emergency room with acute chest pain. Journal of internal medicine 1994;235(2):153-61.
152. Catella F, Healy D, Lawson JA, FitzGerald GA. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. Proceedings of the National Academy of Sciences of the United States of America 1986;83(16):5861-5.
153. Westlund P, Granstrom E, Kumlin M, Nordenstrom A. Identification of 11-dehydro-TXB2 as a suitable parameter for monitoring thromboxane production in the human. Prostaglandins 1986;31(5):929-60.
154. Westlund P, Kumlin M, Nordenstrom A, Granstrom E. Circulating and urinary thromboxane B2 metabolites in the rabbit: 11-dehydro-thromboxane B2 as parameter of thromboxane production. Prostaglandins 1986;31(3):413-43.
155. Morita E, Schroder JM, Christophers E. Identification of a novel and highly potent eosinophil chemotactic lipid in human eosinophils treated with arachidonic acid. Journal of immunology 1990;144(5):1893-900.
156. Powell WS, Gravel S, MacLeod RJ, Mills E, Hashefi M. Stimulation of human neutrophils by 5-oxo-6,8,11,14-eicosatetraenoic acid by a mechanism independent of the leukotriene B4 receptor. The Journal of biological chemistry 1993;268(13):9280-6.
157. Shak S, Perez HD, Goldstein IM. A novel dioxygenation product of arachidonic acid possesses potent chemotactic activity for human polymorphonuclear leukocytes. The Journal of biological chemistry 1983;258(24):14948-53.
158. Hajjar DP, Marcus AJ, Etingin OR. Platelet-neutrophil-smooth muscle cell interactions: lipoxygenase-derived mono- and dihydroxy acids activate cholesteryl ester hydrolysis by the cyclic AMP dependent protein kinase cascade. Biochemistry 1989;28(22):8885-91.
159. Dodge W, Thomas M. The effect of 5-hydroxyeicosatetraenoic acid on the proliferation of granulocyte progenitors and embryonic fibroblasts of the chick. Biochemical and biophysical research communications 1985;131(2):731-5.
160. Goetzl EJ, Weller PF, Sun FF. The regulation of human eosinophil function by endogenous mono-hydroxy-eicosatetraenoic acids (HETEs). Journal of immunology 1980;124(2):926-33.
161. Goetzl EJ, Brash AR, Tauber AI, Oates JA, Hubbard WC. Modulation of human neutrophil function by monohydroxy-eicosatetraenoic acids. Immunology 1980;39(4):491-501.
162. Valone FH, Franklin M, Sun FF, Goetzl EJ. Alveolar macrophage lipoxygenase products of arachidonic acid: isolation and recognition as the predominant constituents of the neutrophil chemotactic activity elaborated by alveolar macrophages. Cell Immunol 1980;54(2):390-401.
163. Stenson WF, Parker CW. Monohydroxyeicosatetraenoic acids (HETEs) induce degranulation of human neutrophils. Journal of immunology 1980;124(5):2100-4.

164. Gordon EE, Gordon JA, Spector AA. HETEs and coronary artery endothelial cells: metabolic and functional interactions. The American journal of physiology 1991;261(4 Pt 1):C623-33.
165. Ghosh J. Rapid induction of apoptosis in prostate cancer cells by selenium: reversal by metabolites of arachidonate 5-lipoxygenase. Biochemical and biophysical research communications 2004;315(3):624-35. doi: 10.1016/j.bbrc.2004.01.100.
166. O'Flaherty JT, Rogers LC, Paumi CM, Hantgan RR, Thomas LR, Clay CE, High K, Chen YQ, Willingham MC, Smitherman PK, et al. 5-Oxo-ETE analogs and the proliferation of cancer cells. Biochimica et biophysica acta 2005;1736(3):228-36. doi: 10.1016/j.bbalip.2005.08.009.
167. Heidel JR, Taylor SM, Laegreid WW, Silflow RM, Liggitt HD, Leid RW. In vivo chemotaxis of bovine neutrophils induced by 5-lipoxygenase metabolites of arachidonic and eicosapentaenoic acid. The American journal of pathology 1989;134(3):671-6.
168. Takenaga M, Hirai A, Terano T, Tamura Y, Kitagawa H, Yoshida S. Comparison of the in vitro effect of eicosapentaenoic acid (EPA)-derived lipoxygenase metabolites on human platelet function with those of arachidonic acid. Thrombosis research 1986;41(3):373-84.
169. Powell WS, Chung D, Gravel S. 5-Oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration. Journal of immunology 1995;154(8):4123-32.
170. O'Flaherty JT, Cordes J, Redman J, Thomas MJ. 5-Oxo-eicosatetraenoate, a potent human neutrophil stimulus. Biochemical and biophysical research communications 1993;192(1):129-34.
171. Armstrong MM, Diaz G, Kenyon V, Holman TR. Inhibitory and mechanistic investigations of oxo-lipids with human lipoxygenase isozymes. Bioorganic & medicinal chemistry 2014;22(15):4293-7. doi: 10.1016/j.bmc.2014.05.025.
172. Yu K, Bayona W, Kallen CB, Harding HP, Ravera CP, McMahon G, Brown M, Lazar MA. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. The Journal of biological chemistry 1995;270(41):23975-83.
173. Brinkman HJ, van Buul-Wortelboer MF, van Mourik JA. Involvement of cyclooxygenase- and lipoxygenase-mediated conversion of arachidonic acid in controlling human vascular smooth muscle cell proliferation. Thrombosis and haemostasis 1990;63(2):291-7.
174. Liu X, Zhang S, Arora JS, Snyder NW, Shah SJ, Blair IA. 11-Oxoeicosatetraenoic acid is a cyclooxygenase-2/15-hydroxyprostaglandin dehydrogenase-derived antiproliferative eicosanoid. Chemical research in toxicology 2011;24(12):2227-36. doi: 10.1021/tx200336f.
175. Nazarewicz RR, Zenebe WJ, Parihar A, Parihar MS, Vaccaro M, Rink C, Sen CK, Ghafourifar P. 12(S)-hydroperoxyeicosatetraenoic acid (12-HETE) increases mitochondrial nitric oxide by increasing intramitochondrial calcium. Archives of biochemistry and biophysics 2007;468(1):114-20. doi: 10.1016/j.abb.2007.09.018.
176. Patricia MK, Kim JA, Harper CM, Shih PT, Berliner JA, Natarajan R, Nadler JL, Hedrick CC. Lipoxygenase products increase monocyte adhesion to human aortic endothelial cells. Arteriosclerosis, thrombosis, and vascular biology 1999;19(11):2615-22.
177. Reilly KB, Srinivasan S, Hatley ME, Patricia MK, Lannigan J, Bolick DT, Vandenhoff G, Pei H, Natarajan R, Nadler JL, et al. 12/15-Lipoxygenase activity mediates

inflammatory monocyte/endothelial interactions and atherosclerosis in vivo. The Journal of biological chemistry 2004;279(10):9440-50. doi: 10.1074/jbc.M303857200.
178. Honn KV, Nelson KK, Renaud C, Bazaz R, Diglio CA, Timar J. Fatty acid modulation of tumor cell adhesion to microvessel endothelium and experimental metastasis. Prostaglandins 1992;44(5):413-29.
179. Sekiya F, Takagi J, Usui T, Kawajiri K, Kobayashi Y, Sato F, Saito Y. 12S-hydroxyeicosatetraenoic acid plays a central role in the regulation of platelet activation. Biochemical and biophysical research communications 1991;179(1):345-51.
180. Sekiya F, Takagi J, Sasaki K, Kawajiri K, Kobayashi Y, Sato F, Saito Y. Feedback regulation of platelet function by 12S-hydroxyeicosatetraenoic acid: inhibition of arachidonic acid liberation from phospholipids. Biochimica et biophysica acta 1990;1044(1):165-8.
181. Fonlupt P, Croset M, Lagarde M. 12-HETE inhibits the binding of PGH2/TXA2 receptor ligands in human platelets. Thrombosis research 1991;63(2):239-48.
182. Croset M, Sala A, Folco G, Lagarde M. Inhibition by lipoxygenase products of TXA2-like responses of platelets and vascular smooth muscle. 14-Hydroxy from 22:6n-3 is more potent than 12-HETE. Biochemical pharmacology 1988;37(7):1275-80.
183. Johnson EN, Brass LF, Funk CD. Increased platelet sensitivity to ADP in mice lacking platelet-type 12-lipoxygenase. Proceedings of the National Academy of Sciences of the United States of America 1998;95(6):3100-5.
184. Tamura Y, Hirai A, Terano T, Takenaga M, Saitoh H, Tahara K, Yoshida S. Clinical and epidemiological studies of eicosapentaenoic acid (EPA) in Japan. Progress in lipid research 1986;25(1-4):461-6.
185. Naccache PH, Leblanc Y, Rokach J, Patrignani P, Fruteau de Laclos B, Borgeat P. Calcium mobilization and right-angle light scatter responses to 12-oxo-derivatives of arachidonic acid in neutrophils: evidence for the involvement of the leukotriene B4 receptor. Biochimica et biophysica acta 1991;1133(1):102-6.
186. Matsuda H, Miyatake K, Dahlen SE. Pharmacodynamics of 15(S)-hydroperoxyeicosatetraenoic (15-HPETE) and 15(S)-hydroxyeicosatetraenoic acid (15-HETE) in isolated arteries from guinea pig, rabbit, rat and human. The Journal of pharmacology and experimental therapeutics 1995;273(3):1182-9.
187. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature 1999;400(6742):378-82. doi: 10.1038/22572.
188. Naruhn S, Meissner W, Adhikary T, Kaddatz K, Klein T, Watzer B, Muller-Brusselbach S, Muller R. 15-hydroxyeicosatetraenoic acid is a preferential peroxisome proliferator-activated receptor beta/delta agonist. Molecular pharmacology 2010;77(2):171-84. doi: 10.1124/mol.109.060541.
189. Takata S, Papayianni A, Matsubara M, Jimenez W, Pronovost PH, Brady HR. 15-Hydroxyeicosatetraenoic acid inhibits neutrophil migration across cytokine-activated endothelium. The American journal of pathology 1994;145(3):541-9.
190. Smith RJ, Justen JM, Nidy EG, Sam LM, Bleasdale JE. Transmembrane signaling in human polymorphonuclear neutrophils: 15(S)-hydroxy-(5Z,8Z,11Z,13E)-eicosatetraenoic acid modulates receptor agonist-triggered cell activation. Proceedings of the National Academy of Sciences of the United States of America 1993;90(15):7270-4.

191. Zhu D, Medhora M, Campbell WB, Spitzbarth N, Baker JE, Jacobs ER. Chronic hypoxia activates lung 15-lipoxygenase, which catalyzes production of 15-HETE and enhances constriction in neonatal rabbit pulmonary arteries. Circulation research 2003;92(9):992-1000. doi: 10.1161/01.RES.0000070881.65194.8F.
192. Setty BN, Werner MH, Hannun YA, Stuart MJ. 15-Hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositide-derived second messengers--sn-1,2-diacylglycerol and inositol-1,4,5-trisphosphate. Blood 1992;80(11):2765-73.
193. Sultana C, Shen Y, Rattan V, Kalra VK. Lipoxygenase metabolites induced expression of adhesion molecules and transendothelial migration of monocyte-like HL-60 cells is linked to protein kinase C activation. Journal of cellular physiology 1996;167(3):477-87. doi: 10.1002/(SICI)1097-4652(199606)167:3<477::AID-JCP12>3.0.CO;2-1.
194. Thollon C, Iliou JP, Cambarrat C, Robin F, Vilaine JP. Nature of the cardiomyocyte injury induced by lipid hydroperoxides. Cardiovascular research 1995;30(5):648-55.
195. Wei C, Zhu P, Shah SJ, Blair IA. 15-oxo-Eicosatetraenoic acid, a metabolite of macrophage 15-hydroxyprostaglandin dehydrogenase that inhibits endothelial cell proliferation. Molecular pharmacology 2009;76(3):516-25. doi: 10.1124/mol.109.057489.
196. Sugumaran PK, Wang S, Song S, Nie X, Zhang L, Feng Y, Ma W, Zhu D. 15-oxo-Eicosatetraenoic acid prevents serum deprivation-induced apoptosis of pulmonary arterial smooth muscle cells by activating pro-survival pathway. Prostaglandins, leukotrienes, and essential fatty acids 2014;90(4):89-98. doi: 10.1016/j.plefa.2014.01.006.
197. Dho S, Grinstein S, Corey EJ, Su WG, Pace-Asciak CR. Hepoxilin A3 induces changes in cytosolic calcium, intracellular pH and membrane potential in human neutrophils. The Biochemical journal 1990;266(1):63-8.
198. Mrsny RJ, Gewirtz AT, Siccardi D, Savidge T, Hurley BP, Madara JL, McCormick BA. Identification of hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. Proceedings of the National Academy of Sciences of the United States of America 2004;101(19):7421-6. doi: 10.1073/pnas.0400832101.
199. Hallenborg P, Jorgensen C, Petersen RK, Feddersen S, Araujo P, Markt P, Langer T, Furstenberger G, Krieg P, Koppen A, et al. Epidermis-type lipoxygenase 3 regulates adipocyte differentiation and peroxisome proliferator-activated receptor gamma activity. Molecular and cellular biology 2010;30(16):4077-91. doi: 10.1128/MCB.01806-08.
200. Hafstrom I, Palmblad J, Malmsten CL, Radmark O, Samuelsson B. Leukotriene B4--a stereospecific stimulator for release of lysosomal enzymes from neutrophils. FEBS letters 1981;130(1):146-8.
201. Ringertz B, Palmblad J, Radmark O, Malmsten C. Leukotriene-induced neutrophil aggregation in vitro. FEBS letters 1982;147(2):180-2.
202. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 1980;286(5770):264-5.
203. Hansson G, Lindgren JA, Dahlen SE, Hedqvist P, Samuelsson B. Identification and biological activity of novel omega-oxidized metabolites of leukotriene B4 from human leukocytes. FEBS letters 1981;130(1):107-12.
204. Palmblad J, Uden AM, Lindgren JA, Radmark O, Hansson G, Malmsten CL. Effects of novel leukotrienes on neutrophil migration. FEBS letters 1982;144(1):81-4.

205. Cheng JB, Lang D, Bewtra A, Townley RG. Tissue distribution and functional correlation of [3H]leukotriene C4 and [3H]leukotriene D4 binding sites in guinea-pig uterus and lung preparations. The Journal of pharmacology and experimental therapeutics 1985;232(1):80-7.
206. Camp RD, Coutts AA, Greaves MW, Kay AB, Walport MJ. Responses of human skin to intradermal injection of leukotrienes C4, D4 and B4. British journal of pharmacology 1983;80(3):497-502.
207. Leitch AG, Lee TH, Ringel EW, Prickett JD, Robinson DR, Pyne SG, Corey EJ, Drazen JM, Austen KF, Lewis RA. Immunologically induced generation of tetraene and pentaene leukotrienes in the peritoneal cavities of menhaden-fed rats. Journal of immunology 1984;132(5):2559-65.
208. Carbajal V, Vargas MH, Flores-Soto E, Martinez-Cordero E, Bazan-Perkins B, Montano LM. LTD4 induces hyperresponsiveness to histamine in bovine airway smooth muscle: role of SR-ATPase Ca2+ pump and tyrosine kinase. American journal of physiology Lung cellular and molecular physiology 2005;288(1):L84-92. doi: 10.1152/ajplung.00446.2003.
209. Ezra D, Boyd LM, Feuerstein G, Goldstein RE. Coronary constriction by leukotriene C4, D4, and E4 in the intact pig heart. The American journal of cardiology 1983;51(8):1451-4.
210. Denis D, Charleson S, Rackham A, Jones TR, Ford-Hutchinson AW, Lord A, Cirino M, Girard Y, Larue M, Rokach J. Synthesis and biological activities of leukotriene F4 and leukotriene F4 sulfone. Prostaglandins 1982;24(6):801-14.
211. Patcha V, Wigren J, Winberg ME, Rasmusson B, Li J, Sarndahl E. Differential inside-out activation of beta2-integrins by leukotriene B4 and fMLP in human neutrophils. Experimental cell research 2004;300(2):308-19. doi: 10.1016/j.yexcr.2004.07.015.
212. Maddox JF, Serhan CN. Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. The Journal of experimental medicine 1996;183(1):137-46.
213. Bannenberg G, Moussignac RL, Gronert K, Devchand PR, Schmidt BA, Guilford WJ, Bauman JG, Subramanyam B, Perez HD, Parkinson JF, et al. Lipoxins and novel 15-epi-lipoxin analogs display potent anti-inflammatory actions after oral administration. British journal of pharmacology 2004;143(1):43-52. doi: 10.1038/sj.bjp.0705912.
214. Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M. A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. The Journal of biological chemistry 2005;280(15):15267-78. doi: 10.1074/jbc.M410638200.
215. Katoh T, Takahashi K, DeBoer DK, Serhan CN, Badr KF. Renal hemodynamic actions of lipoxins in rats: a comparative physiological study. The American journal of physiology 1992;263(3 Pt 2):F436-42.
216. Nigam S, Fiore S, Luscinskas FW, Serhan CN. Lipoxin A4 and lipoxin B4 stimulate the release but not the oxygenation of arachidonic acid in human neutrophils: dissociation between lipid remodeling and adhesion. Journal of cellular physiology 1990;143(3):512-23. doi: 10.1002/jcp.1041430316.
217. Badr KF, DeBoer DK, Schwartzberg M, Serhan CN. Lipoxin A4 antagonizes cellular and in vivo actions of leukotriene D4 in rat glomerular mesangial cells: evidence for

competition at a common receptor. Proceedings of the National Academy of Sciences of the United States of America 1989;86(9):3438-42.
218. Stahl GL, Tsao P, Lefer AM, Ramphal JY, Nicolaou KC. Pharmacologic profile of lipoxins A5 and B5: new biologically active eicosanoids. European journal of pharmacology 1989;163(1):55-60.
219. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. The Journal of experimental medicine 2004;200(1):69-78. doi: 10.1084/jem.20040566.
220. Nascimento-Silva V, Arruda MA, Barja-Fidalgo C, Fierro IM. Aspirin-triggered lipoxin A4 blocks reactive oxygen species generation in endothelial cells: a novel antioxidative mechanism. Thrombosis and haemostasis 2007;97(1):88-98.
221. Maddox JF, Hachicha M, Takano T, Petasis NA, Fokin VV, Serhan CN. Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor. The Journal of biological chemistry 1997;272(11):6972-8.
222. Hercule HC, Schunck WH, Gross V, Seringer J, Leung FP, Weldon SM, da Costa Goncalves A, Huang Y, Luft FC, Gollasch M. Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice. Arteriosclerosis, thrombosis, and vascular biology 2009;29(1):54-60. doi: 10.1161/ATVBAHA.108.171298.
223. Lu T, Katakam PV, VanRollins M, Weintraub NL, Spector AA, Lee HC. Dihydroxyeicosatrienoic acids are potent activators of Ca(2+)-activated K(+) channels in isolated rat coronary arterial myocytes. The Journal of physiology 2001;534(Pt 3):651-67.
224. Oltman CL, Weintraub NL, VanRollins M, Dellsperger KC. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. Circulation research 1998;83(9):932-9.
225. Fang X, Kaduce TL, Weintraub NL, VanRollins M, Spector AA. Functional implications of a newly characterized pathway of 11,12-epoxyeicosatrienoic acid metabolism in arterial smooth muscle. Circulation research 1996;79(4):784-93.
226. Fang X, Hu S, Xu B, Snyder GD, Harmon S, Yao J, Liu Y, Sangras B, Falck JR, Weintraub NL, et al. 14,15-Dihydroxyeicosatrienoic acid activates peroxisome proliferator-activated receptor-alpha. American journal of physiology Heart and circulatory physiology 2006;290(1):H55-63. doi: 10.1152/ajpheart.00427.2005.
227. Panigrahy D, Edin ML, Lee CR, Huang S, Bielenberg DR, Butterfield CE, Barnes CM, Mammoto A, Mammoto T, Luria A, et al. Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. The Journal of clinical investigation 2012;122(1):178-91. doi: 10.1172/JCI58128.
228. Proctor KG, Falck JR, Capdevila J. Intestinal vasodilation by epoxyeicosatrienoic acids: arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. Circulation research 1987;60(1):50-9.
229. Pozzi A, Macias-Perez I, Abair T, Wei S, Su Y, Zent R, Falck JR, Capdevila JH. Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. The Journal of biological chemistry 2005;280(29):27138-46. doi: 10.1074/jbc.M501730200.

230. Zhang Y, Oltman CL, Lu T, Lee HC, Dellsperger KC, VanRollins M. EET homologs potently dilate coronary microvessels and activate BK(Ca) channels. American journal of physiology Heart and circulatory physiology 2001;280(6):H2430-40.
231. Dhanasekaran A, Gruenloh SK, Buonaccorsi JN, Zhang R, Gross GJ, Falck JR, Patel PK, Jacobs ER, Medhora M. Multiple antiapoptotic targets of the PI3K/Akt survival pathway are activated by epoxyeicosatrienoic acids to protect cardiomyocytes from hypoxia/anoxia. American journal of physiology Heart and circulatory physiology 2008;294(2):H724-35. doi: 10.1152/ajpheart.00979.2007.
232. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 1999;285(5431):1276-9.
233. Zhang G, Panigrahy D, Mahakian LM, Yang J, Liu JY, Stephen Lee KS, Wettersten HI, Ulu A, Hu X, Tam S, et al. Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proceedings of the National Academy of Sciences of the United States of America 2013;110(16):6530-5. doi: 10.1073/pnas.1304321110.
234. Campbell WB, Deeter C, Gauthier KM, Ingraham RH, Falck JR, Li PL. 14,15-Dihydroxyeicosatrienoic acid relaxes bovine coronary arteries by activation of K(Ca) channels. American journal of physiology Heart and circulatory physiology 2002;282(5):H1656-64. doi: 10.1152/ajpheart.00597.2001.
235. Terashvili M, Tseng LF, Wu HE, Narayanan J, Hart LM, Falck JR, Pratt PF, Harder DR. Antinociception produced by 14,15-epoxyeicosatrienoic acid is mediated by the activation of beta-endorphin and met-enkephalin in the rat ventrolateral periaqueductal gray. J Pharmacol Exp Ther 2008;326(2):614-22. doi: 10.1124/jpet.108.136739.
236. Carroll MA, Balazy M, Margiotta P, Huang DD, Falck JR, McGiff JC. Cytochrome P-450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides. The American journal of physiology 1996;271(4 Pt 2):R863-9.
237. Bednar MM, Gross CE, Russell SR, Fuller SP, Ahern TP, Howard DB, Falck JR, Reddy KM, Balazy M. 16(R)-hydroxyeicosatetraenoic acid, a novel cytochrome P450 product of arachidonic acid, suppresses activation of human polymorphonuclear leukocyte and reduces intracranial pressure in a rabbit model of thromboembolic stroke. Neurosurgery 2000;47(6):1410-8; discussion 8-9.
238. Carroll MA, Garcia MP, Falck JR, McGiff JC. Cyclooxygenase dependency of the renovascular actions of cytochrome P450-derived arachidonate metabolites. The Journal of pharmacology and experimental therapeutics 1992;260(1):104-9.
239. Ma YH, Gebremedhin D, Schwartzman ML, Falck JR, Clark JE, Masters BS, Harder DR, Roman RJ. 20-Hydroxyeicosatetraenoic acid is an endogenous vasoconstrictor of canine renal arcuate arteries. Circulation research 1993;72(1):126-36.
240. Escalante B, Falck JR, Yadagiri P, Sun LM, Laniado-Schwartzman M. 19(S)-hydroxyeicosatetraenoic acid is a potent stimulator of renal Na+-K+-ATPase. Biochemical and biophysical research communications 1988;152(3):1269-74.
241. Randriamboavonjy V, Busse R, Fleming I. 20-HETE-induced contraction of small coronary arteries depends on the activation of Rho-kinase. Hypertension 2003;41(3 Pt 2):801-6. doi: 10.1161/01.HYP.0000047240.33861.6B.
242. Ishizuka T, Cheng J, Singh H, Vitto MD, Manthati VL, Falck JR, Laniado-Schwartzman M. 20-Hydroxyeicosatetraenoic acid stimulates nuclear factor-kappaB activation and the

production of inflammatory cytokines in human endothelial cells. The Journal of pharmacology and experimental therapeutics 2008;324(1):103-10. doi: 10.1124/jpet.107.130336.
243. Uddin MR, Muthalif MM, Karzoun NA, Benter IF, Malik KU. Cytochrome P-450 metabolites mediate norepinephrine-induced mitogenic signaling. Hypertension 1998;31(1 Pt 2):242-7.
244. Niculescu LS, Sanda GM, Sima AV. HDL inhibits endoplasmic reticulum stress by stimulating apoE and CETP secretion from lipid-loaded macrophages. Biochemical and biophysical research communications 2013;434(1):173-8. doi: 10.1016/j.bbrc.2013.03.050.
245. Hampel JK, Brownrigg LM, Vignarajah D, Croft KD, Dharmarajan AM, Bentel JM, Puddey IB, Yeap BB. Differential modulation of cell cycle, apoptosis and PPARgamma2 gene expression by PPARgamma agonists ciglitazone and 9-hydroxyoctadecadienoic acid in monocytic cells. Prostaglandins, leukotrienes, and essential fatty acids 2006;74(5):283-93. doi: 10.1016/j.plefa.2006.03.002.
246. Hattori T, Obinata H, Ogawa A, Kishi M, Tatei K, Ishikawa O, Izumi T. G2A plays proinflammatory roles in human keratinocytes under oxidative stress as a receptor for 9-hydroxyoctadecadienoic acid. The Journal of investigative dermatology 2008;128(5):1123-33. doi: 10.1038/sj.jid.5701172.
247. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell 1998;93(2):229-40.
248. Buchanan MR, Haas TA, Lagarde M, Guichardant M. 13-Hydroxyoctadecadienoic acid is the vessel wall chemorepellant factor, LOX. The Journal of biological chemistry 1985;260(30):16056-9.
249. Tloti MA, Moon DG, Weston LK, Kaplan JE. Effect of 13-hydroxyoctadeca-9,11-dienoic acid (13-HODE) on thrombin induced platelet adherence to endothelial cells in vitro. Thrombosis research 1991;62(4):305-17.
250. Miller CC, Ziboh VA. Induction of epidermal hyperproliferation by topical n-3 polyunsaturated fatty acids on guinea pig skin linked to decreased levels of 13-hydroxyoctadecadienoic acid (13-hode). The Journal of investigative dermatology 1990;94(3):353-8.
251. Murthy S, Born E, Mathur S, Field FJ. 13-hydroxy octadecadienoic acid (13-HODE) inhibits triacylglycerol-rich lipoprotein secretion by CaCo-2 cells. Journal of lipid research 1998;39(6):1254-62.
252. De Meyer GR, Bult H, Verbeuren TJ, Herman AG. The role of endothelial cells in the relaxations induced by 13-hydroxy- and 13-hydroperoxylinoleic acid in canine arteries. British journal of pharmacology 1992;107(2):597-603.
253. Altmann R, Hausmann M, Spottl T, Gruber M, Bull AW, Menzel K, Vogl D, Herfarth H, Scholmerich J, Falk W, et al. 13-Oxo-ODE is an endogenous ligand for PPARgamma in human colonic epithelial cells. Biochemical pharmacology 2007;74(4):612-22. doi: 10.1016/j.bcp.2007.05.027.
254. Edin ML, Wang Z, Bradbury JA, Graves JP, Lih FB, DeGraff LM, Foley JF, Torphy R, Ronnekleiv OK, Tomer KB, et al. Endothelial expression of human cytochrome P450 epoxygenase CYP2C8 increases susceptibility to ischemia-reperfusion injury in isolated

mouse heart. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2011;25(10):3436-47. doi: 10.1096/fj.11-188300.
255. Moran JH, Weise R, Schnellmann RG, Freeman JP, Grant DF. Cytotoxicity of linoleic acid diols to renal proximal tubular cells. Toxicology and applied pharmacology 1997;146(1):53-9. doi: 10.1006/taap.1997.8197.
256. Zheng J, Plopper CG, Lakritz J, Storms DH, Hammock BD. Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. American journal of respiratory cell and molecular biology 2001;25(4):434-8. doi: 10.1165/ajrcmb.25.4.4104.
257. Nowak G, Grant DF, Moran JH. Linoleic acid epoxide promotes the maintenance of mitochondrial function and active Na+ transport following hypoxia. Toxicology letters 2004;147(2):161-75.
258. Sakai T, Ishizaki T, Ohnishi T, Sasaki F, Ameshima S, Nakai T, Miyabo S, Matsukawa S, Hayakawa M, Ozawa T. Leukotoxin, 9,10-epoxy-12-octadecenoate inhibits mitochondrial respiration of isolated perfused rat lung. The American journal of physiology 1995;269(3 Pt 1):L326-31.
259. Ozawa T, Hayakawa M, Takamura T, Sugiyama S, Suzuki K, Iwata M, Taki F, Tomita T. Biosynthesis of leukotoxin, 9,10-epoxy-12 octadecenoate, by leukocytes in lung lavages of rat after exposure to hyperoxia. Biochemical and biophysical research communications 1986;134(3):1071-8.
260. Sugiyama S, Hayakawa M, Nagai S, Ajioka M, Ozawa T. Leukotoxin, 9, 10-epoxy-12-octadecenoate, causes cardiac failure in dogs. Life sciences 1987;40(3):225-31.
261. Ozawa T, Nishikimi M, Sugiyama S, Taki F, Hayakawa M, Shionoya H. Cytotoxic activity of leukotoxin, a neutrophil-derived fatty acid epoxide, on cultured human cells. Biochemistry international 1988;16(2):369-73.
262. Siegfried MR, Aoki N, Lefer AM, Elisseou EM, Zipkin RE. Direct cardiovascular actions of two metabolites of linoleic acid. Life sciences 1990;46(6):427-33.
263. Moran JH, Nowak G, Grant DF. Analysis of the toxic effects of linoleic acid, 12,13-cis-epoxyoctadecenoic acid, and 12,13-dihydroxyoctadecenoic acid in rabbit renal cortical mitochondria. Toxicology and applied pharmacology 2001;172(2):150-61. doi: 10.1006/taap.2001.9149.
264. Schroder R, Xue L, Konya V, Martini L, Kampitsch N, Whistler JL, Ulven T, Heinemann A, Pettipher R, Kostenis E. PGH1, the precursor for the anti-inflammatory prostaglandins of the 1-series, is a potent activator of the pro-inflammatory receptor CRTH2/DP2. PloS one 2012;7(3):e33329. doi: 10.1371/journal.pone.0033329.
265. De Caridi G, Massara M, Stilo F, Spinelli F, Grande R, Butrico L, de Franciscis S, Serra R. Effectiveness of prostaglandin E1 in patients with mixed arterial and venous ulcers of the lower limbs. International wound journal 2014. doi: 10.1111/iwj.12334.
266. Natsume T, Iwatsuki K, Nishizuka T, Arai T, Yamamoto M, Hirata H. Prostaglandin E1 alleviates neuropathic pain and neural dysfunction from entrapment neuropathy associated with diabetes mellitus. Microsurgery 2014;34(7):568-75. doi: 10.1002/micr.22281.
267. Ney P, Feelisch M. Vasodilator effects of PGE1 in the coronary and systemic circulation of the rat are mediated by ATP-sensitive potassium (K+) channels. Agents and actions Supplements 1995;45:71-6.
268. Makino H, Aoki M, Hashiya N, Yamasaki K, Hiraoka K, Shimizu H, Azuma J, Kurinami H, Ogihara T, Morishita R. Increase in peripheral blood flow by intravenous

administration of prostaglandin E1 in patients with peripheral arterial disease, accompanied by up-regulation of hepatocyte growth factor. Hypertension research : official journal of the Japanese Society of Hypertension 2004;27(2):85-91.
269. Zhang CY, Ma ZS, Ma LL, Wang LX. Effect of prostaglandin E1 inhalation on pulmonary hypertension following corrective surgery for congenital heart disease. Experimental and clinical cardiology 2013;18(1):13-6.
270. Westwick J. The effect of pulmonary metabolites of prostaglandins E1, E2 and F2alpha on ADP-induced aggregation of human and rabbit platelets [proceedings]. British journal of pharmacology 1976;58(2):297P-8P.
271. Conners MS, Schwartzman ML, Quan X, Heilman E, Chauhan K, Falck JR, Godfrey HP. Enhancement of delayed hypersensitivity inflammatory reactions in guinea pig skin by 12(R)-hydroxy-5,8,14-eicosatrienoic acid. The Journal of investigative dermatology 1995;104(1):47-51.
272. Ikei KN, Yeung J, Apopa PL, Ceja J, Vesci J, Holman TR, Holinstat M. Investigations of human platelet-type 12-lipoxygenase: role of lipoxygenase products in platelet activation. Journal of lipid research 2012;53(12):2546-59. doi: 10.1194/jlr.M026385.
273. Ziboh VA, Miller CC, Cho Y. Significance of lipoxygenase-derived monohydroxy fatty acids in cutaneous biology. Prostaglandins & other lipid mediators 2000;63(1-2):3-13.
274. Vang K, Ziboh VA. 15-lipoxygenase metabolites of gamma-linolenic acid/eicosapentaenoic acid suppress growth and arachidonic acid metabolism in human prostatic adenocarcinoma cells: possible implications of dietary fatty acids. Prostaglandins, leukotrienes, and essential fatty acids 2005;72(5):363-72. doi: 10.1016/j.plefa.2005.02.002.
275. Schulze-Tanzil G, de SP, Behnke B, Klingelhoefer S, Scheid A, Shakibaei M. Effects of the antirheumatic remedy hox alpha--a new stinging nettle leaf extract--on matrix metalloproteinases in human chondrocytes in vitro. Histology and histopathology 2002;17(2):477-85.
276. Durot I, Devillard L, Tissier C, Vandroux D, Voisin S, Jaquir S, Rochette L, Athias P. Dependence on the phospholipid polyunsaturated fatty acids of the oxidative injury of isolated cardiomyocytes. Free radical research 2006;40(3):251-61. doi: 10.1080/10715760500509165.
277. Takahashi H, Hara H, Goto T, Kamakari K, Wataru N, Mohri S, Takahashi N, Suzuki H, Shibata D, Kawada T. 13-Oxo-9(Z),11(E),15(Z)-octadecatrienoic Acid Activates Peroxisome Proliferator-Activated Receptor gamma in Adipocytes. Lipids 2015;50(1):3-12. doi: 10.1007/s11745-014-3972-x.
278. Lefils-Lacourtablaise J, Socorro M, Geloen A, Daira P, Debard C, Loizon E, Guichardant M, Dominguez Z, Vidal H, Lagarde M, et al. The eicosapentaenoic acid metabolite 15-deoxy-delta(12,14)-prostaglandin J3 increases adiponectin secretion by adipocytes partly via a PPARgamma-dependent mechanism. PLoS One 2013;8(5):e63997. doi: 10.1371/journal.pone.0063997.
279. Kulkarni PS, Srinivasan BD. Prostaglandins E3 and D3 lower intraocular pressure. Investigative ophthalmology & visual science 1985;26(8):1178-82.
280. Wendling MG, DuCharme DW. Cardiovascular effects of prostaglandin D3 and D2 in anesthetized dogs. Prostaglandins 1981;22(2):235-43.
281. Hemker DP, Aiken JW. Effects of prostaglandin D3 on nerve transmission in nictitating membrane of cats. European journal of pharmacology 1980;67(1):155-8.

282. Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proceedings of the National Academy of Sciences of the United States of America 2003;100(4):1751-6. doi: 10.1073/pnas.0334211100.
283. Yang P, Chan D, Felix E, Cartwright C, Menter DG, Madden T, Klein RD, Fischer SM, Newman RA. Formation and antiproliferative effect of prostaglandin E(3) from eicosapentaenoic acid in human lung cancer cells. Journal of lipid research 2004;45(6):1030-9. doi: 10.1194/jlr.M300455-JLR200.
284. Xia S, Lu Y, Wang J, He C, Hong S, Serhan CN, Kang JX. Melanoma growth is reduced in fat-1 transgenic mice: impact of omega-6/omega-3 essential fatty acids. Proceedings of the National Academy of Sciences of the United States of America 2006;103(33):12499-504. doi: 10.1073/pnas.0605394103.
285. Shimizu T, Yokotani K. Effects of centrally administered prostaglandin E(3) and thromboxane A(3) on plasma noradrenaline and adrenaline in rats: comparison with prostaglandin E(2) and thromboxane A(2). European journal of pharmacology 2009;611(1-3):30-4. doi: 10.1016/j.ejphar.2009.03.057.
286. Faust TW, Lee E, Redfern JS, Feldman M. Effect of prostaglandin F3 alpha on gastric mucosal injury by ethanol in rats: comparison with prostaglandin F2 alpha. Prostaglandins 1989;37(4):493-504.
287. Kobzar G, Mardla V, Jarving I, Samel N. Comparison of anti-aggregatory effects of PGI2, PGI3 and iloprost on human and rabbit platelets. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2001;11(5):279-84. doi: 47814.
288. Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci U S A 1979;76(2):944-8.
289. Hegde S, Kaushal N, Ravindra KC, Chiaro C, Hafer KT, Gandhi UH, Thompson JT, van den Heuvel JP, Kennett MJ, Hankey P, et al. Delta12-prostaglandin J3, an omega-3 fatty acid-derived metabolite, selectively ablates leukemia stem cells in mice. Blood 2011;118(26):6909-19. doi: 10.1182/blood-2010-11-317750.
290. Kogure R, Toyama K, Hiyamuta S, Kojima I, Takeda S. 5-Hydroxy-eicosapentaenoic acid is an endogenous GPR119 agonist and enhances glucose-dependent insulin secretion. Biochemical and biophysical research communications 2011;416(1-2):58-63. doi: 10.1016/j.bbrc.2011.10.141.
291. Fujita T, Sakuma S, Yamamoto N, Fujimoto Y. Effects of eicosapentaenoic acid and its 15-hydroperoxy and 15-hydroxy derivatives on glucosamine synthetase activity in rabbit gastric mucosa. Biochemistry and molecular biology international 1998;46(1):157-63.
292. Sakuma S, Usa K, Fujimoto Y. 15-Hydroperoxyeicosapentaenoic acid, but not eicosapentaenoic acid, shifts arachidonic acid away from cyclooxygenase pathway into acyl-CoA synthetase pathway in rabbit kidney medulla microsomes. Prostaglandins, leukotrienes, and essential fatty acids 2006;75(2):69-74. doi: 10.1016/j.plefa.2006.06.003.
293. Tsunomori M, Fujimoto Y, Muta E, Nishida H, Sakuma S, Fujita T. 15-Hydroperoxyeicosapentaenoic acid inhibits arachidonic acid metabolism in rabbit platelets more potently than eicosapentaenoic acid. Biochimica et biophysica acta 1996;1300(3):171-6.

294. Nathaniel DJ, Evans JF, Leblanc Y, Leveille C, Fitzsimmons BJ, Ford-Hutchinson AW. Leukotriene A5 is a substrate and an inhibitor of rat and human neutrophil LTA4 hydrolase. Biochemical and biophysical research communications 1985;131(2):827-35.
295. Juan H, Peskar BA, Simmet T. Effect of exogenous 5,8,11,14,17-eicosapentaenoic acid on cardiac anaphylaxis. British journal of pharmacology 1987;90(2):315-25.
296. Hammarstrom S. Leukotriene C5: a slow reacting substance derived from eicosapentaenoic acid. The Journal of biological chemistry 1980;255(15):7093-4.
297. Ait-Said F, Elalamy I, Werts C, Gomard MT, Jacquemin C, Couetil JP, Hatmi M. Inhibition by eicosapentaenoic acid of IL-1beta-induced PGHS-2 expression in human microvascular endothelial cells: involvement of lipoxygenase-derived metabolites and p38 MAPK pathway. Biochimica et biophysica acta 2003;1631(1):77-84.
298. Lauritzen L, Hoffmann EK, Hansen HS, Jensen B. Dietary n-3 and n-6 fatty acids are equipotent in stimulating volume regulation in Ehrlich ascites tumor cells. The American journal of physiology 1993;264(1 Pt 1):C109-17.
299. Lam BK, Wong PY. Biosynthesis and biological activities of lipoxin A5 and B5 from eicosapentaenoic acid. Adv Exp Med Biol 1988;229:51-9.
300. VanRollins M. Epoxygenase metabolites of docosahexaenoic and eicosapentaenoic acids inhibit platelet aggregation at concentrations below those affecting thromboxane synthesis. The Journal of pharmacology and experimental therapeutics 1995;274(2):798-804.
301. Jung F, Schulz C, Blaschke F, Muller DN, Mrowietz C, Franke RP, Lendlein A, Schunck WH. Effect of cytochrome P450-dependent epoxyeicosanoids on Ristocetin-induced thrombocyte aggregation. Clinical hemorheology and microcirculation 2012;52(2-4):403-16. doi: 10.3233/CH-2012-1614.
302. Morin C, Sirois M, Echave V, Rizcallah E, Rousseau E. Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. American journal of physiology Lung cellular and molecular physiology 2009;296(1):L130-9. doi: 10.1152/ajplung.90436.2008.
303. Morin C, Sirois M, Echave V, Albadine R, Rousseau E. 17,18-epoxyeicosatetraenoic acid targets PPARgamma and p38 mitogen-activated protein kinase to mediate its anti-inflammatory effects in the lung: role of soluble epoxide hydrolase. American journal of respiratory cell and molecular biology 2010;43(5):564-75. doi: 10.1165/rcmb.2009-0155OC.
304. Hercule HC, Salanova B, Essin K, Honeck H, Falck JR, Sausbier M, Ruth P, Schunck WH, Luft FC, Gollasch M. The vasodilator 17,18-epoxyeicosatetraenoic acid targets the pore-forming BK alpha channel subunit in rodents. Experimental physiology 2007;92(6):1067-76. doi: 10.1113/expphysiol.2007.038166.
305. Endo J, Sano M, Isobe Y, Fukuda K, Kang JX, Arai H, Arita M. 18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload-induced maladaptive cardiac remodeling. The Journal of experimental medicine 2014;211(8):1673-87. doi: 10.1084/jem.20132011.
306. Weylandt KH, Krause LF, Gomolka B, Chiu CY, Bilal S, Nadolny A, Waechter SF, Fischer A, Rothe M, Kang JX. Suppressed liver tumorigenesis in fat-1 mice with elevated omega-3 fatty acids is associated with increased omega-3 derived lipid mediators and reduced TNF-alpha. Carcinogenesis 2011;32(6):897-903. doi: 10.1093/carcin/bgr049.

307. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. The Journal of experimental medicine 2005;201(5):713-22. doi: 10.1084/jem.20042031.
308. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN. Molecular circuits of resolution: formation and actions of resolvins and protectins. Journal of immunology 2005;174(7):4345-55.
309. Qiu W, Guo K, Yi L, Gong Y, Huang L, Zhong W. Resolvin E1 reduces hepatic fibrosis in mice with infection. Experimental and therapeutic medicine 2014;7(6):1481-5. doi: 10.3892/etm.2014.1641.
310. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 2007;447(7146):869-74. doi: 10.1038/nature05877.
311. Tjonahen E, Oh SF, Siegelman J, Elangovan S, Percarpio KB, Hong S, Arita M, Serhan CN. Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis. Chemistry & biology 2006;13(11):1193-202. doi: 10.1016/j.chembiol.2006.09.011.
312. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 formation and impact in inflammation resolution. Journal of immunology 2012;188(9):4527-34. doi: 10.4049/jimmunol.1103652.
313. Ogawa S, Urabe D, Yokokura Y, Arai H, Arita M, Inoue M. Total synthesis and bioactivity of resolvin E2. Organic letters 2009;11(16):3602-5. doi: 10.1021/ol901350g.
314. Isobe Y, Arita M, Iwamoto R, Urabe D, Todoroki H, Masuda K, Inoue M, Arai H. Stereochemical assignment and anti-inflammatory properties of the omega-3 lipid mediator resolvin E3. Journal of biochemistry 2013;153(4):355-60. doi: 10.1093/jb/mvs151.
315. Lu Y, Tian H, Hong S. Novel 14,21-dihydroxy-docosahexaenoic acids: structures, formation pathways, and enhancement of wound healing. Journal of lipid research 2010;51(5):923-32. doi: 10.1194/jlr.M000059.
316. Tian H, Lu Y, Shah SP, Hong S. Novel 14S,21-dihydroxy-docosahexaenoic acid rescues wound healing and associated angiogenesis impaired by acute ethanol intoxication/exposure. Journal of cellular biochemistry 2010;111(2):266-73. doi: 10.1002/jcb.22709.
317. Sapieha P, Stahl A, Chen J, Seaward MR, Willett KL, Krah NM, Dennison RJ, Connor KM, Aderman CM, Liclican E, et al. 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids. Science translational medicine 2011;3(69):69ra12. doi: 10.1126/scitranslmed.3001571.
318. Gonzalez-Periz A, Planaguma A, Gronert K, Miquel R, Lopez-Parra M, Titos E, Horrillo R, Ferre N, Deulofeu R, Arroyo V, et al. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2006;20(14):2537-9. doi: 10.1096/fj.06-6250fje.
319. Li X, Hong S, Li PL, Zhang Y. Docosahexanoic acid-induced coronary arterial dilation: actions of 17S-hydroxy docosahexanoic acid on K+ channel activity. The Journal of pharmacology and experimental therapeutics 2011;336(3):891-9. doi: 10.1124/jpet.110.176461.

320. Lima-Garcia JF, Dutra RC, da Silva K, Motta EM, Campos MM, Calixto JB. The precursor of resolvin D series and aspirin-triggered resolvin D1 display anti-hyperalgesic properties in adjuvant-induced arthritis in rats. British journal of pharmacology 2011;164(2):278-93. doi: 10.1111/j.1476-5381.2011.01345.x.
321. Bento AF, Claudino RF, Dutra RC, Marcon R, Calixto JB. Omega-3 fatty acid-derived mediators 17(R)-hydroxy docosahexaenoic acid, aspirin-triggered resolvin D1 and resolvin D2 prevent experimental colitis in mice. Journal of immunology 2011;187(4):1957-69. doi: 10.4049/jimmunol.1101305.
322. Gleissman H, Yang R, Martinod K, Lindskog M, Serhan CN, Johnsen JI, Kogner P. Docosahexaenoic acid metabolome in neural tumors: identification of cytotoxic intermediates. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2010;24(3):906-15. doi: 10.1096/fj.09-137919.
323. Abdulnour RE, Dalli J, Colby JK, Krishnamoorthy N, Timmons JY, Tan SH, Colas RA, Petasis NA, Serhan CN, Levy BD. Maresin 1 biosynthesis during platelet-neutrophil interactions is organ-protective. Proceedings of the National Academy of Sciences of the United States of America 2014;111(46):16526-31. doi: 10.1073/pnas.1407123111.
324. Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2012;26(4):1755-65. doi: 10.1096/fj.11-201442.
325. Gong J, Wu ZY, Qi H, Chen L, Li HB, Li B, Yao CY, Wang YX, Wu J, Yuan SY, et al. Maresin 1 mitigates LPS-induced acute lung injury in mice. British journal of pharmacology 2014;171(14):3539-50. doi: 10.1111/bph.12714.
326. Duffield JS, Hong S, Vaidya VS, Lu Y, Fredman G, Serhan CN, Bonventre JV. Resolvin D series and protectin D1 mitigate acute kidney injury. Journal of immunology 2006;177(9):5902-11.
327. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proceedings of the National Academy of Sciences of the United States of America 2004;101(22):8491-6. doi: 10.1073/pnas.0402531101.
328. Chen P, Vericel E, Lagarde M, Guichardant M. Poxytrins, a class of oxygenated products from polyunsaturated fatty acids, potently inhibit blood platelet aggregation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2011;25(1):382-8. doi: 10.1096/fj.10-161836.
329. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, et al. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem 2003;278(44):43807-17. doi: 10.1074/jbc.M305841200.
330. Liu M, Boussetta T, Makni-Maalej K, Fay M, Driss F, El-Benna J, Lagarde M, Guichardant M. Protectin DX, a double lipoxygenase product of DHA, inhibits both ROS production in human neutrophils and cyclooxygenase activities. Lipids 2014;49(1):49-57. doi: 10.1007/s11745-013-3863-6.
331. White PJ, St-Pierre P, Charbonneau A, Mitchell PL, St-Amand E, Marcotte B, Marette A. Protectin DX alleviates insulin resistance by activating a myokine-liver glucoregulatory axis. Nature medicine 2014;20(6):664-9. doi: 10.1038/nm.3549.

332. Hiram R, Rizcallah E, Sirois C, Sirois M, Morin C, Fortin S, Rousseau E. Resolvin D1 reverses reactivity and Ca2+ sensitivity induced by ET-1, TNF-alpha, and IL-6 in the human pulmonary artery. American journal of physiology Heart and circulatory physiology 2014;307(11):H1547-58. doi: 10.1152/ajpheart.00452.2014.
333. Chen F, Fan XH, Wu YP, Zhu JL, Wang F, Bo LL, Li JB, Bao R, Deng XM. Resolvin D1 improves survival in experimental sepsis through reducing bacterial load and preventing excessive activation of inflammatory response. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 2014;33(3):457-64. doi: 10.1007/s10096-013-1978-6.
334. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA, Flower RJ, Perretti M, Serhan CN. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature 2009;461(7268):1287-91. doi: 10.1038/nature08541.
335. Park CK, Xu ZZ, Liu T, Lu N, Serhan CN, Ji RR. Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. The Journal of neuroscience : the official journal of the Society for Neuroscience 2011;31(50):18433-8. doi: 10.1523/JNEUROSCI.4192-11.2011.
336. Bohr S, Patel SJ, Sarin D, Irimia D, Yarmush ML, Berthiaume F. Resolvin D2 prevents secondary thrombosis and necrosis in a mouse burn wound model. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2013;21(1):35-43. doi: 10.1111/j.1524-475X.2012.00853.x.
337. Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CY, Chiang N, Petasis NA, Serhan CN. Resolvin D3 and aspirin-triggered resolvin D3 are potent immunoresolvents. Chemistry & biology 2013;20(2):188-201. doi: 10.1016/j.chembiol.2012.11.010.
338. Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Serhan CN. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature 2012;484(7395):524-8. doi: 10.1038/nature11042.
339. Ye D, Zhang D, Oltman C, Dellsperger K, Lee HC, VanRollins M. Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. The Journal of pharmacology and experimental therapeutics 2002;303(2):768-76.
340. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arteriosclerosis, thrombosis, and vascular biology 2011;31(5):986-1000. doi: 10.1161/ATVBAHA.110.207449.
341. Schneider C, Pozzi A. Cyclooxygenases and lipoxygenases in cancer. Cancer metastasis reviews 2011;30(3-4):277-94. doi: 10.1007/s10555-011-9310-3.
342. Dogne JM, Hanson J, Pratico D. Thromboxane, prostacyclin and isoprostanes: therapeutic targets in atherogenesis. Trends in pharmacological sciences 2005;26(12):639-44. doi: 10.1016/j.tips.2005.10.001.
343. Tateson JE, Moncada S, Vane JR. Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. Prostaglandins 1977;13(3):389-97.
344. Svensson J, Hamberg M, Samuelsson B. On the formation and effects of thromboxane A2 in human platelets. Acta physiologica Scandinavica 1976;98(3):285-94. doi: 10.1111/j.1748-1716.1976.tb10313.x.
345. Eklund B, Carlson LA. Central and peripheral circulatory effects and metabolic effects of different prostaglandins given I.V. to man. Prostaglandins 1980;20(2):333-47.

346. Iyu D, Juttner M, Glenn JR, White AE, Johnson AJ, Fox SC, Heptinstall S. PGE1 and PGE2 modify platelet function through different prostanoid receptors. Prostaglandins & other lipid mediators 2011;94(1-2):9-16. doi: 10.1016/j.prostaglandins.2010.11.001.
347. Needleman P, Minkes M, Raz A. Thromboxanes: selective biosynthesis and distinct biological properties. Science 1976;193(4248):163-5.
348. Walton LJ, Franklin IJ, Bayston T, Brown LC, Greenhalgh RM, Taylor GW, Powell JT. Inhibition of prostaglandin E2 synthesis in abdominal aortic aneurysms: implications for smooth muscle cell viability, inflammatory processes, and the expansion of abdominal aortic aneurysms. Circulation 1999;100(1):48-54.
349. Marcus AJ, Weksler BB, Jaffe EA. Enzymatic conversion of prostaglandin endoperoxide H2 and arachidonic acid to prostacyclin by cultured human endothelial cells. The Journal of biological chemistry 1978;253(20):7138-41.
350. Uderhardt S, Kronke G. 12/15-lipoxygenase during the regulation of inflammation, immunity, and self-tolerance. Journal of molecular medicine 2012;90(11):1247-56. doi: 10.1007/s00109-012-0954-4.
351. Martinez-Clemente M, Claria J, Titos E. The 5-lipoxygenase/leukotriene pathway in obesity, insulin resistance, and fatty liver disease. Current opinion in clinical nutrition and metabolic care 2011;14(4):347-53. doi: 10.1097/MCO.0b013e32834777fa.
352. Poeckel D, Funk CD. The 5-lipoxygenase/leukotriene pathway in preclinical models of cardiovascular disease. Cardiovascular research 2010;86(2):243-53. doi: 10.1093/cvr/cvq016.
353. Ardaillou R, Baud L, Sraer J. Leukotrienes and other lipoxygenase products of arachidonic acid synthesized in the kidney. The American journal of medicine 1986;81(2B):12-22.
354. Menna C, Olivieri F, Catalano A, Procopio A. Lipoxygenase inhibitors for cancer prevention: promises and risks. Current pharmaceutical design 2010;16(6):725-33.
355. Aharony D, Smith JB, Silver MJ. Regulation of arachidonate-induced platelet aggregation by the lipoxygenase product, 12-hydroperoxyeicosatetraenoic acid. Biochimica et biophysica acta 1982;718(2):193-200.
356. Katoh A, Ikeda H, Murohara T, Haramaki N, Ito H, Imaizumi T. Platelet-derived 12-hydroxyeicosatetraenoic acid plays an important role in mediating canine coronary thrombosis by regulating platelet glycoprotein IIb/IIIa activation. Circulation 1998;98(25):2891-8.
357. Valone FH, Franklin M, Sun FF, Goetzl EJ. Alveolar Macrophage Lipoxygenase Products of Arachidonic-Acid - Isolation and Recognition as the Predominant Constituents of the Neutrophil Chemotactic Activity Elaborated by Alveolar Macrophages. Cell Immunol 1980;54(2):390-401. doi: Doi 10.1016/0008-8749(80)90219-1.
358. Goetzl EJ, Woods JM, Gorman RR. Stimulation of human eosinophil and neutrophil polymorphonuclear leukocyte chemotaxis and random migration by 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid. The Journal of clinical investigation 1977;59(1):179-83. doi: 10.1172/JCI108617.
359. Powell WS, Hashefi M, Falck JR, Chauhan K, Rokach J, Wang SS, Mills E, MacLeod RJ. Effects of oxo and dihydro metabolites of 12-hydroxy-5,8,10,14-eicosatetraenoic acid on chemotaxis and cytosolic calcium levels in human neutrophils. J Leukoc Biol 1995;57(2):257-63.

360. Samuelsson B. Leukotrienes: mediators of allergic reactions and inflammation. International archives of allergy and applied immunology 1981;66 Suppl 1:98-106.
361. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nature reviews Immunology 2008;8(5):349-61. doi: 10.1038/nri2294.
362. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proceedings of the National Academy of Sciences of the United States of America 1995;92(21):9475-9.
363. Serhan CN, Maddox JF, Petasis NA, Akritopoulouzanze I, Papayianni A, Brady HR, Colgan SP, Madara JL. Design of Lipoxin a(4) Stable Analogs That Block Transmigration and Adhesion of Human Neutrophils. Biochemistry 1995;34(44):14609-15. doi: Doi 10.1021/Bi00044a041.
364. Ku G, Thomas CE, Akeson AL, Jackson RL. Induction of interleukin 1 beta expression from human peripheral blood monocyte-derived macrophages by 9-hydroxyoctadecadienoic acid. The Journal of biological chemistry 1992;267(20):14183-8.
365. Feldstein AE, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, Zhang R, McIntyre TM, Hazen SL. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Journal of lipid research 2010;51(10):3046-54. doi: 10.1194/jlr.M007096.
366. Yoshida Y, Yoshikawa A, Kinumi T, Ogawa Y, Saito Y, Ohara K, Yamamoto H, Imai Y, Niki E. Hydroxyoctadecadienoic acid and oxidatively modified peroxiredoxins in the blood of Alzheimer's disease patients and their potential as biomarkers. Neurobiol Aging 2009;30(2):174-85. doi: DOI 10.1016/j.neurobiolaging.2007.06.012.
367. Shureiqi I, Wojno KJ, Poore JA, Reddy RG, Moussalli MJ, Spindler SA, Greenson JK, Normolle D, Hasan AA, Lawrence TS, et al. Decreased 13-S-hydroxyoctadecadienoic acid levels and 15-lipoxygenase-1 expression in human colon cancers. Carcinogenesis 1999;20(10):1985-95.
368. Tabolacci C, Lentini A, Provenzano B, Gismondi A, Rossi S, Beninati S. Similar antineoplastic effects of nimesulide, a selective COX-2 inhibitor, and prostaglandin E1 on B16-F10 murine melanoma cells. Melanoma research 2010;20(4):273-9. doi: 10.1097/CMR.0b013e328339d8ac.
369. Wang X, Lin H, Gu Y. Multiple roles of dihomo-gamma-linolenic acid against proliferation diseases. Lipids in health and disease 2012;11:25. doi: 10.1186/1476-511X-11-25.
370. Ziboh VA, Yun M, Hyde DM, Giri SN. gamma-Linolenic acid-containing diet attenuates bleomycin-induced lung fibrosis in hamsters. Lipids 1997;32(7):759-67.
371. Evans JF, Nathaniel DJ, Zamboni RJ, Ford-Hutchinson AW. Leukotriene A3. A poor substrate but a potent inhibitor of rat and human neutrophil leukotriene A4 hydrolase. The Journal of biological chemistry 1985;260(20):10966-70.
372. Evans J, Zamboni R, Nathaniel D, Leveille C, Ford-Hutchinson AW. Characterization of biological properties of synthetic and biological leukotriene B3. Prostaglandins 1985;30(6):981-8.
373. Campbell WB, Fleming I. Epoxyeicosatrienoic acids and endothelium-dependent responses. Pflugers Archiv : European journal of physiology 2010;459(6):881-95. doi: 10.1007/s00424-010-0804-6.

374. McGiff JC, Quilley J. 20-HETE and the kidney: resolution of old problems and new beginnings. The American journal of physiology 1999;277(3 Pt 2):R607-23.
375. Salmon ED, Goode D, Maugel TK, Bonar DB. Pressure-induced depolymerization of spindle microtubules. III. Differential stability in HeLa cells. The Journal of cell biology 1976;69(2):443-54.
376. Kaduce TL, Fang X, Harmon SD, Oltman CL, Dellsperger KC, Teesch LM, Gopal VR, Falck JR, Campbell WB, Weintraub NL, et al. 20-hydroxyeicosatetraenoic acid (20-HETE) metabolism in coronary endothelial cells. J Biol Chem 2004;279(4):2648-56. doi: 10.1074/jbc.M306849200.
377. Fang X, Faraci FM, Kaduce TL, Harmon S, Modrick ML, Hu S, Moore SA, Falck JR, Weintraub NL, Spector AA. 20-Hydroxyeicosatetraenoic acid is a potent dilator of mouse basilar artery: role of cyclooxygenase. Am J Physiol Heart Circ Physiol 2006;291(5):H2301-7. doi: 10.1152/ajpheart.00349.2006.
378. Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. Hypertension 2002;39(2 Pt 2):690-4.
379. Bellien J, Joannides R. Epoxyeicosatrienoic acid pathway in human health and diseases. Journal of cardiovascular pharmacology 2013;61(3):188-96. doi: 10.1097/FJC.0b013e318273b007.
380. Ramirez CE, Shuey MM, Milne GL, Gilbert K, Hui N, Yu C, Luther JM, Brown NJ. Arg287Gln variant of EPHX2 and epoxyeicosatrienoic acids are associated with insulin sensitivity in humans. Prostaglandins & other lipid mediators 2014;113-115:38-44. doi: 10.1016/j.prostaglandins.2014.08.001.
381. Ozawa T, Sugiyama S, Hayakawa M, Satake T, Taki F, Iwata M, Taki K. Existence of leukotoxin 9,10-epoxy-12-octadecenoate in lung lavages from rats breathing pure oxygen and from patients with the adult respiratory distress syndrome. The American review of respiratory disease 1988;137(3):535-40. doi: 10.1164/ajrccm/137.3.535.
382. Zhang W, Nagao M, Takatori T, Iwadate K, Itakura Y, Yamada Y, Iwase H, Oono T. Immunohistochemical dynamics of leukotoxin (9,10-epoxy-12-octadecenoic acid) in lungs of rats. International journal of legal medicine 1995;107(4):174-8.
383. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. Nat Med 1997;3(5):562-6.
384. Ozawa T, Sugiyama S, Hayakawa M, Taki F, Hanaki Y. Neutrophil microsomes biosynthesize linoleate epoxide (9,10-epoxy-12-octadecenoate), a biological active substance. Biochemical and biophysical research communications 1988;152(3):1310-8.
385. Hawcroft G, Loadman PM, Belluzzi A, Hull MA. Effect of eicosapentaenoic acid on E-type prostaglandin synthesis and EP4 receptor signaling in human colorectal cancer cells. Neoplasia 2010;12(8):618-27.
386. Kramer HJ, Stevens J, Grimminger F, Seeger W. Fish oil fatty acids and human platelets: dose-dependent decrease in dienoic and increase in trienoic thromboxane generation. Biochem Pharmacol 1996;52(8):1211-7.
387. Wang W, Zhu J, Lyu F, Panigrahy D, Ferrara KW, Hammock B, Zhang G. omega-3 Polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer. Prostaglandins & other lipid mediators 2014. doi: 10.1016/j.prostaglandins.2014.07.002.

388. Miller AM, van Bekkum DW, Kobb SM, McCrohan MB, Knaan-Shanzer S. Dietary fish oil supplementation alters LTB4:LTB5 ratios but does not affect the expression of acute graft versus host disease in mice. Prostaglandins, leukotrienes, and essential fatty acids 1993;49(2):561-8.
389. Mickleborough TD, Lindley MR, Ionescu AA, Fly AD. Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. Chest 2006;129(1):39-49. doi: 10.1378/chest.129.1.39.
390. Lee TH, Menica-Huerta JM, Shih C, Corey EJ, Lewis RA, Austen KF. Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B5 and the double lipoxygenase product. The Journal of biological chemistry 1984;259(4):2383-9.
391. Leitinger N, Tyner TR, Oslund L, Rizza C, Subbanagounder G, Lee H, Shih PT, Mackman N, Tigyi G, Territo MC, et al. Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. Proceedings of the National Academy of Sciences of the United States of America 1999;96(21):12010-5.
392. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. The Journal of experimental medicine 2009;206(1):15-23. doi: 10.1084/jem.20081880.
393. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, Petasis NA, Serhan CN. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proceedings of the National Academy of Sciences of the United States of America 2010;107(4):1660-5. doi: 10.1073/pnas.0907342107.
394. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol 2006;176(3):1848-59.
395. Chen P, Fenet B, Michaud S, Tomczyk N, Vericel E, Lagarde M, Guichardant M. Full characterization of PDX, a neuroprotectin/protectin D1 isomer, which inhibits blood platelet aggregation. FEBS letters 2009;583(21):3478-84. doi: 10.1016/j.febslet.2009.10.004.
396. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. Blood 2008;112(3):848-55. doi: 10.1182/blood-2007-11-122598.
397. Morita M, Kuba K, Ichikawa A, Nakayama M, Katahira J, Iwamoto R, Watanebe T, Sakabe S, Daidoji T, Nakamura S, et al. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 2013;153(1):112-25. doi: 10.1016/j.cell.2013.02.027.
398. Imai Y. Role of omega-3 PUFA-derived mediators, the protectins, in influenza virus infection. Biochim Biophys Acta 2015;1851(4):496-502. doi: 10.1016/j.bbalip.2015.01.006.
399. Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta 2015;1851(4):397-413. doi: 10.1016/j.bbalip.2014.08.006.

400. Lauterbach B, Barbosa-Sicard E, Wang MH, Honeck H, Kargel E, Theuer J, Schwartzman ML, Haller H, Luft FC, Gollasch M, et al. Cytochrome P450-dependent eicosapentaenoic acid metabolites are novel BK channel activators. Hypertension 2002;39(2 Pt 2):609-13.
401. Fischer R, Konkel A, Mehling H, Blossey K, Gapelyuk A, Wessel N, von Schacky C, Dechend R, Muller DN, Rothe M, et al. Dietary Omega-3 Fatty Acids Modulate the Eicosanoid Profile in Man Primarily via the CYP-epoxygenase Pathway. Journal of lipid research 2014. doi: 10.1194/jlr.M047357.
402. Arnold C, Markovic M, Blossey K, Wallukat G, Fischer R, Dechend R, Konkel A, von Schacky C, Luft FC, Muller DN, et al. Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of {omega}-3 fatty acids. The Journal of biological chemistry 2010;285(43):32720-33. doi: 10.1074/jbc.M110.118406.
403. Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, Petasis NA, Levy BD, Serhan CN, Van Dyke TE. RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2006;20(2):401-3. doi: 10.1096/fj.05-4724fje.
404. Serhan CN. Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotectins. Current opinion in clinical nutrition and metabolic care 2005;8(2):115-21.
405. 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.
406. Quehenberger O, Dennis EA. The human plasma lipidome. The New England journal of medicine 2011;365(19):1812-23. doi: 10.1056/NEJMra1104901.
407. Nording ML, Yang J, Georgi K, Hegedus Karbowski C, German JB, Weiss RH, Hogg RJ, Trygg J, Hammock BD, Zivkovic AM. Individual variation in lipidomic profiles of healthy subjects in response to omega-3 Fatty acids. PloS one 2013;8(10):e76575. doi: 10.1371/journal.pone.0076575.
408. Schuchardt JP, Schneider I, Willenberg I, Yang J, Hammock BD, Hahn A, Schebb NH. Increase of EPA-derived hydroxy, epoxy and dihydroxy fatty acid levels in human plasma after a single dose of long-chain omega-3 PUFA. Prostaglandins & other lipid mediators 2014;109-111:23-31. doi: 10.1016/j.prostaglandins.2014.03.001.
409. Shearer GC, Harris WS, Pedersen TL, Newman JW. Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters. Journal of lipid research 2010;51(8):2074-81. doi: 10.1194/M900193-JLR200.
410. Keenan AH, Pedersen TL, Fillaus K, Larson MK, Shearer GC, Newman JW. Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers. Journal of lipid research 2012;53(8):1662-9. doi: 10.1194/jlr.P025577.
411. Lundstrom SL, Yang J, Brannan JD, Haeggstrom JZ, Hammock BD, Nair P, O'Byrne P, Dahlen SE, Wheelock CE. Lipid mediator serum profiles in asthmatics significantly shift following dietary supplementation with omega-3 fatty acids. Mol Nutr Food Res 2013;57(8):1378-89. doi: DOI 10.1002/mnfr.201200827.

412. Ramsden CE, Ringel A, Feldstein AE, Taha AY, MacIntosh BA, Hibbeln JR, Majchrzak-Hong SF, Faurot KR, Rapoport SI, Cheon Y, et al. Lowering dietary linoleic acid reduces bioactive oxidized linoleic acid metabolites in humans. Prostag Leukotr Ess 2012;87(4-5):135-41. doi: DOI 10.1016/j.plefa.2012.08.004.
413. Caligiuri SP, Aukema HM, Ravandi A, Guzman R, Dibrov E, Pierce GN. Flaxseed consumption reduces blood pressure in patients with hypertension by altering circulating oxylipins via an alpha-linolenic acid-induced inhibition of soluble epoxide hydrolase. Hypertension 2014;64(1):53-9. doi: 10.1161/HYPERTENSIONAHA.114.03179.
414. Caligiuri SP, Aukema HM, Ravandi A, Pierce GN. Elevated levels of pro-inflammatory oxylipins in older subjects are normalized by flaxseed consumption. Experimental gerontology 2014. doi: 10.1016/j.exger.2014.04.005.
415. Calder PC, Deckelbaum RJ. Harmful, harmless or helpful? The n-6 fatty acid debate goes on. Current opinion in clinical nutrition and metabolic care 2011;14(2):113-4. doi: 10.1097/MCO.0b013e328343d895.
416. Lagarde M, Bernoud-Hubac N, Guichardant M. Expanding the horizons of lipidomics. Towards fluxolipidomics. Mol Membr Biol 2012;29(7):222-8. doi: 10.3109/09687688.2012.689378.
417. Lagarde M, Bernoud-Hubac N, Calzada C, Vericel E, Guichardant M. Lipidomics of essential fatty acids and oxygenated metabolites. Mol Nutr Food Res 2013;57(8):1347-58. doi: 10.1002/mnfr.201200828.
418. Matsunobu T, Okuno T, Yokoyama C, Yokomizo T. Thromboxane A synthase-independent production of 12-hydroxyheptadecatrienoic acid, a BLT2 ligand. J Lipid Res 2013;54(11):2979-87. doi: 10.1194/jlr.M037754.

4.2 Protocol 1 - Blood Processing Protocol

Day 0 and 28

Five tubes of blood were collected from participants on Day 0 and Day 28: 4.5 mL LiHEP tube, 10.0 mL EDTA tube, 4.0 mL EDTA tube, two 5.0 mL SST Serum tubes, and one urine cup. The LiHep tube was immediately sent to St Boniface Hospital Biochemistry to process the lipid panel.

Plasma EDTA tubes were centrifuged at 1100 g for 10 min at 4°C. Samples were aliquoted for oxylipins, fatty acids, and other analytes. Antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added at 3.3% to plasma set aside for oxylipin and fatty acid analysis. Any additional plasma was aliqouted into labeled centrifuge tubes. Buffy coat was removed from 10.0 mL EDTA tube and spun down at 1100 g for 10 min at 4°C. Red blood cells were collected from EDTA tubes, washed with 0.09% normal saline and centrifuged at 650 g for 10 min at 4°C, two times, and one time at 1500g for 10 min at 4°C. RBC were suspended in phosphate-buffered saline (pH 7.4). All samples were stored in boxes at -80°C until further analyzed.

Serum SST tubes were incubated at room temperature for 30-45 minutes and then centrifuged at 1170 g for 10 minutes at room temperature. Samples were aliquoted for oxylipins, fatty acids, and metabolomics. Antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added at 3.3% to serum set aside for oxylipin and fatty acid analysis and protease inhibitor was added at 1.0% to serum set aside for metabolomics analysis. All samples were stored in boxes at -80°C until further analysis.

Urine was aliquoted for oxylipin analysis and metabolomics analysis. Antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added at 0.33% to samples set aside for oxylipin analysis. All samples were stored in boxes at -80°C until analyzed.

Day 1, 3, 7, and 14

Two tubes of blood were collected from participants on each Day 1, 3, 7, and 14: 10.0 mL EDTA tube and 4.0 mL EDTA tube, and one urine cup. Plasma EDTA tubes were centrifuged at 1100 g for 10 min at 4°C. Samples were aliqouted for oxylipins, fatty acids, and other analyses. Antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added at 3.3% to plasma set aside for oxylipin and fatty acid analysis. Any additional plasma was aliqouted into labeled centrifuge tubes. All samples were stored in boxes at -80°C until analyzed.

Urine was aliquoted for oxylipin analysis and metabolomics analysis. Antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added at 0.33% to samples set aside for oxylipin analysis. All samples were stored in boxes at -80°C until analyzed.

4.3 Protocol 2 – Plasma Fatty Acid Protocol

To complete plasma fatty acid analysis a total lipid extraction was conducted, followed by methylation, and analyzed by GC. Practice samples were prepared to optimize the correct volume of internal standard to use.

In glass tubes that have been cleaned with Contrad solution, 250 µl of prepared plasma and 2.5 mL 2:1 chloroform:methanol with 0.01% BHT (0.03 g BHT, 200 mL chloroform, 100 mL methanol) were added. Samples were vortexed and 100 µL of the 1,2-dipentadecanoyl-*sn*-

glycero-3-phosphocholine (1.8 mg/mL in chloroform) internal standard [Avanti Polar Lipids, Inc. catalogue no. 850350] was added to each sample.

Further, 2.25 mL of chloroform:methanol was added to the sample, the samples were vortexed, and then 950 µL 0.73% sodium chloride was added. Samples were then centrifuged at 4^0C for 10 minutes at 800 g. The lower phase of the sample was then removed using a Pasteur pipette and transferred into a Contrad cleaned 12.0 mL glass vial. Next, samples were dried down in a nitrogen evaporator water bath at 37^0C and once dried, 1.2 mL methanolic sulfuric acid (6.0 mL sulfuric acid, 94.0 mL methanol) was added to each sample. Samples were then tightly capped and placed in an 88^0C oven for 1.5 hours.

Next, 1.5 mL toluene was added to the samples and vortexed. Then, 1.0 mL ultrapure water was added to the samples, vortexed, and centrifuged at 800 g for 5 minutes. The top layer of the samples was transferred to a clean 2.0 mL GC vial. Samples were then dried down in a nitrogen evaporator water bath at 37^0C and reconstituted with 50 µL hexane. Samples were stored at -20^0C until analyzed by GC.

Samples were separated on a DB225MS column (30 m X 0.25 mm diameter and 0.25 mm film thickness; Agilent Technologies Canada Inc., Mississauga, Ontario) using a Bruker 450-GC with flame ionization detector. The temperature program was 70^0C for 1 min, raised to 180^0C at 25^0C/min and held for 1 minute, raised to 200^0C at 10^0C/min and held for 1 min, and raised to 220^0C at 2^0C/min and held for 6 min. Finally, the temperature was raised to 240^0C at 20^0C/min and held for 20 minutes. Total run time was 46:40 minutes with a 20:1 split ratio and a column flow of 1.3 mL/min. Hydrogen was used as the carrier gas. Peaks were quantified using the internal standard (C15:0 - Phospholipids) and values are expressed as nmol/L of plasma.

4.4 Protocol 3 – Dietary Oil Fatty Acid Protocol

To complete fatty acid analysis of investigational oils, a total lipid extraction was performed followed by methylation, and analysis by GC. Practice samples were prepared to optimize the correct volume of internal standard to use.

Ahead of analysis, each capsule was weighed and all lipids were extracted from them. This was done by placing capsule in a scintillation vial and covering with approximately 3.0 mL hexane. The capsule outer coating was pierced and deconstructed using a surgical scalpel; the coating and utensils were washed with hexane, saving the wash with the extracted oil sample. Samples were then dried down in a nitrogen evaporator water bath at 37^0C leaving only the lipid contents of the capsule.

In glass tubes that have been cleaned with Contrad solution, 0.11 g of oil was weighed and added to 1.0 mL of hexane. Samples were vortexed and 10 µL of heptadecanoic acid internal standard [(5.5 mg/mL in chloroform) Nu Check Prep Inc., catalogue no. U-17-A], as the standard for triacylglycerides, was added to each sample and vortexed. Next, 100 uL of the diluted oil was transferred into a new glass tube that was cleaned with Contrad solution and dried down in a nitrogen evaporator water bath at 37^0C.

Once dried, 1.0 mL of toluene was added and samples were vortexed. Next, 1.2 mL methanolic sulfuric acid (6.0 mL sulfuric acid, 94.0 mL methanol) was added to each sample and vortexed. Samples were then tightly capped and placed in an 80^0C oven for 60 minutes. Once cooled, 1.0 mL ultrapure water and 1.0 mL hexane were added to the samples, vortexed, and centrifuged at 800 g for 5 minutes. The top layer of the samples was transferred to a new glass tube that was cleaned with Contrad solution and to it 2.0 mL of ultra pure water was added, vortexed and centrifuged at 800 g for 5 minutes. Approximately 1.0 mL of the top phase of the

biphasic mixture was transferred to a clean 2.0 mL GC vial. Samples were stored at -20^{0}C until analyzed by GC.

Samples were separated on a DB225MS column (30 m X 0.25 mm diameter and 0.25 mm film thickness; Agilent Technologies Canada Inc., Mississauga, Ontario) using a Bruker 450-GC with flame ionization detector. The temperature program was 70^{0}C for 1 min, raised to 180^{0}C at 25^{0}C/min and held for 1 minute, raised to 200^{0}C at 10^{0}C/min and held for 1 min, and raised to 220^{0}C at 2^{0}C/min and held for 6 min. Finally, temperature was raised to 240^{0}C at 20^{0}C/min and held for 20 minutes. Total run time was 46:40 minutes with a 20:1 split ratio and a column flow of 1.3 mL/min. Hydrogen was used as the carrier gas. Peaks were quantified using the internal standard (C17:0 - Triglycerides) and values are expressed as % fatty acid per capsule.

4.5 Protocol 4 - Oxylipin Protocol

Oxylipins were analyzed using HPLC-MS/MS on a Luna 5µm C18(2) 100Å 250 x 2.0 mm column. To begin, 400 µl of prepared plasma or serum were added to 1.0 mL of water at pH3. To this, 30 µL of internal standard was added to each centrifuge tube and vortexed. Internal Standard composition is listed in Table 0-1. Samples were then acidified to pH3, vortexed, and centrifuged for 5 min at 3000 rpm at 4^{0}C.

Solid phase extraction was preformed using a Strata-X SPE (Phenomenex) (33 u, 60 mg/3 mL) column for each sample. Each column was pre-conditioned with 2.0 mL 100% methanol and 2.0 mL water at pH3. Sample supernatant was applied to the column and allowed to drip through by gravity. Meanwhile, 1.0 mL of 10% methanol in water at pH3 was added to the sample vial for rinse. This was then vortexed, centrifuged at 4°C, 3000 rpm for 5 minutes, and applied to column. After allowing the rinse to drip through by gravity, the sample was then

pushed through to dry the column and the sample was then eluted into new 1.5 mL microfuge tubes with 1.0 ml 100% methanol. Samples were stored at -80^{0}C until analyzed by HPLC-MS/MS.

When ready to run on HPLC, samples were dried down in a nitrogen evaporator bath at 37^{0}C and reconstituted in 100 µL Solvent A (water-acetonitrile-formic acid [70:30:0.02 v/v/v]. Samples were then vortexed and centrifuged at 14000 g for 10 minutes at 4^{0}C then transferred into labeled GC vials containing a 200 µL polypropylene conical insert. Samples were run on a Shimadzu Nexera XR HPLC and ABSciex QTRAP 6500 MS with triple quadrapole electrospray ionization (IonDrive Turbo V). Anthough slightly different for each analyte, the limit of detection was in the region of 0.12 pg and the limit of quantification was in the region 0.14 pg. Samples were analyzed using MultiQuant Version 3.0. The analyte peak areas were divided by internal standard, normalized to the detector response factor, and converted to nM. The collision-induced dissociation (CID) mass transitions and detector response factors are provided in **Table 0-2**.

Table 0-1.Volume and Concentration of Deuterated Internal Standards

Oxylipin	Volume Added (µL)	Stock Concentration (ng/µL)	Internal Standard Final Concentration (ng/uL)
6-k-$PGF_{1\alpha}$-d4	30.0	25	0.75
TXB_2-d4	20.0	25	0.50
$PGF_{2\alpha}$-d4	20.0	50	1.00
PGE_2-d4	10.0	50	0.50
PGD_2-d4	50.0	25	1.25
13,14-dihydro-15-keto-$PGF_{2\alpha}$	20.0	50	1.00
LTB_4-d4	80.0	25	2.00
20-HETE-d6	80.0	25	2.00
15-HETE-d8	40.0	25	1.00
5-HETE-d8	80.0	25	2.00
13-HODE-d4	40.0	25	1.00
9-HODE-d4	40.0	25	1.00
12,13-DiHOME-d4	20.0	25	0.50
9,10-DiHOME-d4	20.0	25	0.50
14,15-DHET-d11	10.0	25	0.25
11,12-DHET-d11	10.0	25	0.25
8,9-DHET-d11	40.0	25	1.00
15d-PGJ_2-d4	80.0	25	2.00
EPA-d5	40.0	50	2.00
5-OxoETE-d7	270.0	25	6.75
Total	1000.0		

Table 0-2: CID Mass Transitions, Surrogate Deuterated Internal Standards, and Detector Response Factors for Oxylipins Detected.

Component Name	CID Mass Transition (Da)	IS Name	Detector Response Factor
9-HODE	295.0 / 171.0	(d4) 9-HODE.IS	1.47
13-HODE	295.0 / 195.0	(d4) 13-HODE.IS	2.95
9-oxoODE	293.0 / 185.0	(d7) 5-oxoETE.IS	1.44
13-oxoODE	293.0 / 167.0	(d7) 5-oxoETE.IS	0.27
9,10 EpOME	295.0 / 171.0	(d4) 9,10 diHOME.IS	3.76
12,13 EpOME	295.0 / 195.0	(d4) 12,13 diHOME.IS	10.52
9,10 diHOME	313.0 / 201.0	(d4) 9,10 diHOME.IS	16.43
12,13 diHOME	313.0 / 183.0	(d4) 12,13 diHOME.IS	11.80
9,10,13 triHOME	329.0 / 171.0	(d4) 9-HODE.IS	3.13
9,12,13 triHOME	329.0 / 211.0	(d4) 9-HODE.IS	6.19
13-HOTrE-γ	293.0 / 193.0	(d4) 13-HODE.IS	1.70
TXB_1	371.0 / 171.0	(d4) TXB2.IS	3.68
$PGF_{1\alpha}$	355.0 / 293.0	(d4) PGF2a.IS	3.07
8-HETrE	321.0 / 157.0	(d8) 5-HETE.IS	1.81
15-HETrE	321.0 / 221.0	(d8) 15-HETE.IS	2.94
6k $PGF_{1\alpha}$	369.0 / 245.0	(d4) 6k PGF1a.IS	3.65
TXB_2	369.0 / 169.0	(d4) TXB2.IS	6.37
$PGF_{2\alpha}$	353.0 / 193.0	(d4) PGF2a.IS	3.42
PGE_2	351.0 / 271.0	(d4) PGE2.IS	7.86
PGD_2	351.0 / 271.0	(d4) PGD2.IS	1.61
5-iso $PGF_{2\alpha}$VI	353.0 / 115.0	(d4) PGF2a.IS	1.58
tetranor 12-HETE	265.0 / 109.0	(d8) 15-HETE.IS	2.04
11bPGE_2	351.0 / 271.0	(d4) PGE2.IS	4.92
12-HHTrE	279.0 / 217.0	(d8) 5-HETE.IS	0.36
5-HETE	319.0 / 115.0	(d8) 5-HETE.IS	1.17
8-HETE	319.0 / 155.0	(d8) 5-HETE.IS	2.31
9-HETE	319.0 / 123.0	(d8) 5-HETE.IS	0.25
11-HETE	319.0 / 167.0	(d8) 5-HETE.IS	5.32
12-HETE	319.0 / 135.0	(d8) 15-HETE.IS	0.19
15-HETE	319.0 / 175.0	(d8) 15-HETE.IS	0.90
16-HETE	319.0 / 189.0	(d8) 15-HETE.IS	0.54
17-HETE	319.0 / 247.0	(d8) 15-HETE.IS	3.08
18-HETE	319.0 / 261.0	(d6) 20-HETE.IS	3.21
20-HETE	319.0 / 245.0	(d6) 20-HETE.IS	0.40
LTB_4	335.0 / 195.0	(d4) LTB4.IS	1.25
20oh LTB_4	351.0 / 195.0	(d4) LTB4.IS	0.61
LTE_4	438.0 / 333.0	(d4) LTB4.IS	0.94
6S-LXA_4	351.0 / 115.0	(d4) LTB4.IS	0.29

5-oxoETE	317.0 / 203.0	(d7) 5-oxoETE.IS	0.85
12-oxoETE	317.0 / 153.0	(d7) 5-oxoETE.IS	1.52
15-oxoETE	317.0 / 113.0	(d7) 5-oxoETE.IS	2.23
5,6 EpETrE	319.0 / 191.0	(d11) 11,12 DiHETrE.IS	3.73
11,12 EpETrE	319.0 / 167.0	(d11) 11,12 DiHETrE.IS	9.13
14,15 EpETrE	319.0 / 175.0	(d11) 14,15 DiHETrE.IS	1.04
5,6 DiHETrE	337.0 / 145.0	(d11) 11,12 DiHETrE.IS	3.61
8,9 DiHETrE	337.0 / 127.0	(d11) 8,9 DiHETrE.IS	5.54
11,12 DiHETrE	337.0 / 167.0	(d11) 11,12 DiHETrE.IS	16.42
14,15 DiHETrE	337.0 / 207.0	(d11) 14,15 DiHETrE.IS	10.94
2,3-dinor TXB_2	341.0 / 137.0	(d4) TXB2.IS	0.36
9-HOTrE	293.0 / 171.0	(d4) 9-HODE.IS	2.01
13-HOTrE	293.4 / 195.0	(d4) 13-HODE.IS	0.62
9 oxoOTrE	291.0 / 185.0	(d7) 5-oxoETE.IS	2.25
12,13 EpODE	293.0 / 183.0	(d4) 13-HODE.IS	2.40
12,13 diHODE	311.0 / 183.0	(d4) 12,13 diHOME.IS	4.44
TXB_3	367.0 / 169.0	(d4) TXB2.IS	5.50
5-HEPE	317.0 / 115.0	(d8) 5-HETE.IS	1.11
8-HEPE	317.0 / 155.0	(d8) 5-HETE.IS	1.17
9-HEPE	317.0 / 149.0	(d8) 5-HETE.IS	0.68
12-HEPE	317.0 / 179.0	(d8) 15-HETE.IS	1.72
15-HEPE	317.0 / 219.0	(d8) 15-HETE.IS	1.87
18-HEPE	317.0 / 215.0	(d6) 20-HETE.IS	1.39
14,15 EpETE	317.0 / 207.0	(d11) 14,15 DiHETrE.IS	2.68
17,18 EpETE	317.0 / 259.0	(d11) 14,15 DiHETrE.IS	1.86
4-HDoHE	343.0 / 101.0	(d8) 5-HETE.IS	0.44
7-HDoHE	343.0 / 141.0	(d8) 5-HETE.IS	0.55
8-HDoHE	343.0 / 109.0	(d8) 5-HETE.IS	0.45
10-HDoHE	343.0 / 153.0	(d8) 5-HETE.IS	2.18
11-HDoHE	343.0 / 149.0	(d8) 5-HETE.IS	0.85
13-HDoHE	343.0 / 221.0	(d8) 15-HETE.IS	0.60
14-HDoHE	343.0 / 205.0	(d8) 15-HETE.IS	0.78
16-HDoHE	343.0 / 233.0	(d8) 15-HETE.IS	3.35
17-HDoHE	343.0 / 245.0	(d8) 15-HETE.IS	0.28
20-HDoHE	343.0 / 241.0	(d6) 20-HETE.IS	1.33
15-oxoEDE	321.0 / 223.0	(d7) 5-oxoETE.IS	1.16
16,17 EpDPE	343.0 / 193.0	(d11) 14,15 DiHETrE.IS	0.24
19,20 EpDPE	343.0 / 241.0	(d11) 14,15 DiHETrE.IS	2.22
19,20 DiHDPE	361.0 / 229.0	(d11) 14,15 DiHETrE.IS	1.13

Table 0-3. CID Mass Transitions, Surrogate Deuterated Internal Standards, and Detector Response Factors for Oxylipins Scanned.

Component Name	CID Mass Transition (Da)	IS Name	Detector Response Factor
10-HDoHE	343.0 / 153.0	(d8) 5-HETE.IS	2.1831
10-Nitrooleate	326.0 / 169.0	(d5) EPA.IS	--
10S,17S-DiHDoHE	359.0 / 153.0	(d4) LTB_4.IS	1.0292
11-HDoHE	343.0 / 149.0	(d8) 5-HETE.IS	0.8501
11-HEPE	317.0 / 215.0	(d8) 5-HETE.IS	--
11-HETE	319.0 / 167.0	(d8) 5-HETE.IS	5.3158
11,12 DiHETrE	337.0 / 167.0	(d11) 11,12 DiHETrE.IS	16.4237
11,12 EpETrE	319.0 / 167.0	(d11) 11,12 DiHETrE.IS	9.1336
11b $PGF_{2\alpha}$	353.0 / 335.0	(d4) $PGF_{2\alpha}$.IS	0.1703
11bdhk $PGF_{2\alpha}$	353.0 / 113.0	(d4) $PGF_{2\alpha}$.IS	0.8673
11βPGE_2	351.0 / 271.0	(d4) PGE_2.IS	4.9206
11d-TXB_2	367.0 / 305.0	(d4) TXB_2.IS	2.3750
12-HEPE	317.0 / 179.0	(d8) 15-HETE.IS	1.7239
12-HETE	319.0 / 135.0	(d8) 15-HETE.IS	0.1890
12-HHTrE	279.0 / 217.0	(d8) 5-HETE.IS	0.3646
12-oxoETE	317.0 / 153.0	(d7) 5-oxoETE.IS	1.5173
12,13 diHODE	311.0 / 183.0	(d4) 12,13 diHOME.IS	4.4379
12,13 diHOME	313.0 / 183.0	(d4) 12,13 diHOME.IS	11.7980
12,13 EpODE	293.0 / 183.0	(d4) 13-HODE.IS	2.4048
12,13 EpOME	295.0 / 195.0	(d4) 12,13 diHOME.IS	10.5160
12epi LTB_4	335.0 / 195.0	(d4) LTB_4.IS	1.4375
12oxo LTB_4	333.0 / 179.0	(d4) LTB_4.IS	1.4903
13 oxoOTrE	291.0 / 247.0	(d7) 5-oxoETE.IS	0.0856
13-HDoHE	343.0 / 221.0	(d8) 15-HETE.IS	0.6030
13-HODE	295.0 / 195.0	(d4) 13-HODE.IS	2.9546
13-HOTrE	293.4 / 195.0	(d4) 13-HODE.IS	0.6216
13-HOTrE-γ	293.0 / 193.0	(d4) 13-HODE.IS	1.6998
13-oxoODE	293.0 / 167.0	(d7) 5-oxoETE.IS	0.2717
14-HDoHE	343.0 / 205.0	(d8) 15-HETE.IS	0.7806
14,15 DiHETrE	337.0 / 207.0	(d11) 14,15 DiHETrE.IS	10.9390
14,15 EpETE	317.0 / 207.0	(d11) 14,15 DiHETrE.IS	2.6835
14,15 EpETrE	319.0 / 175.0	(d11) 14,15 DiHETrE.IS	1.0437
14,15-LTC_4	624.0 / 272.0	(d4) LTB_4.IS	--
14,15-LTD_4	495.0 / 177.0	(d4) LTB_4.IS	--
14,15-LTE_4	438.0 / 333.0	(d4) LTB_4.IS	--
15-HEPE	317.0 / 219.0	(d8) 15-HETE.IS	1.8731
15-HETE	319.0 / 175.0	(d8) 15-HETE.IS	0.9018
15-HETrE	321.0 / 221.0	(d8) 15-HETE.IS	2.9424

15-oxoEDE	321.0 / 223.0	(d7) 5-oxoETE.IS	1.1612
15-oxoETE	317.0 / 113.0	(d7) 5-oxoETE.IS	2.2341
15,16 diHODE	311.0 / 223.0	(d4) 12,13 diHOME.IS	--
15,16 EpODE	293.0 / 235.0	(d4) 12,13 diHOME.IS	--
15d PGA_2	315.0 / 271.0	(d4) 15d PGJ_2.IS	2.6609
15d PGD_2	333.0 / 271.0	(d4) 15d PGJ_2.IS	29.7220
15d PGJ_2	315.0 / 203.0	(d4) 15d PGJ_2.IS	1.4436
15k PGD_2	349.0 / 235.0	(d4) PGD_2.IS	--
15k PGE_2	349.0 / 235.0	(d4) PGE_2.IS	1.3377
15k $PGF_{1\alpha}$	353.0 / 221.0	(d4) $PGF_{2\alpha}$.IS	--
15k $PGF_{2\alpha}$	351.0 / 219.0	(d4) $PGF_{2\alpha}$.IS	0.6609
15R-LXA_4	351.0 / 165.0	(d4) LTB_4.IS	--
15t PD1	359.0 / 153.0	(d4) LTB_4.IS	--
16-HDoHE	343.0 / 233.0	(d8) 15-HETE.IS	3.3544
16-HETE	319.0 / 189.0	(d8) 15-HETE.IS	0.5383
16,17 EpDPE	343.0 / 193.0	(d11) 14,15 DiHETrE.IS	0.2435
17-HDoHE	343.0 / 245.0	(d8) 15-HETE.IS	0.2779
17-HETE	319.0 / 247.0	(d8) 15-HETE.IS	3.0768
17,18 EpETE	317.0 / 259.0	(d11) 14,15 DiHETrE.IS	1.8647
17k DHA	341.0 / 297.0	(d4) LTB_4.IS	1.1412
17k DPA	343.0 / 247.0	(d4) LTB_4.IS	0.6190
18-HEPE	317.0 / 215.0	(d6) 20-HETE.IS	1.3858
18-HETE	319.0 / 261.0	(d6) 20-HETE.IS	3.2074
19-HETE	319.0 / 231.0	(d6) 20-HETE.IS	0.2715
19,20 DiHDoPE	361.0 / 229.0	(d11) 14,15 DiHETrE.IS	1.1322
19,20 EpDPE	343.0 / 241.0	(d11) 14,15 DiHETrE.IS	2.2213
19oh PGE_2	367.0 / 243.0	(d4) PGE_2.IS	0.3835
19oh $PGF_{2\alpha}$	369.0 / 192.0	(d4) $PGF_{2\alpha}$.IS	--
2,3-dinor 11β $PGF_{2\alpha}$	325.0 / 227.0	(d4) $PGF_{2\alpha}$.IS	1.2716
2,3-dinor 8-iso $PGF_{2\alpha}$	325.0 / 237.0	(d4) $PGF_{2\alpha}$.IS	5.3857
2,3-dinor TXB_2	341.0 / 137.0	(d4) TXB_2.IS	0.3628
2,3-dinor-6k $PGF_{1\alpha}$	363.0 / 281.0	(d4) $PGF_{2\alpha}$.IS	--
20-HDoHE	343.0 / 241.0	(d6) 20-HETE.IS	1.3316
20-HETE	319.0 / 245.0	(d6) 20-HETE.IS	0.3998
20cooh AA	333.0 / 271.0	(d5) EPA.IS	1.1049
20cooh LTB_4	365.0 / 195.0	(d4) LTB_4.IS	0.1691
20oh LTB_4	351.0 / 195.0	(d4) LTB_4.IS	0.6127
20oh PGE_2	367.0 / 175.0	(d4) PGE_2.IS	--
20oh $PGF_{2\alpha}$	369.0 / 165.0	(d4) $PGF_{2\alpha}$.IS	--
4-HDoHE	343.0 / 101.0	(d8) 5-HETE.IS	0.4409
5-HEPE	317.0 / 115.0	(d8) 5-HETE.IS	1.1101
5-HETE	319.0 / 115.0	(d8) 5-HETE.IS	1.1690

5-HETrE	321.0 / 205.0	(d8) 5-HETE.IS	0.2746
5-iso $PGF_{2\alpha}$VI	353.0 / 115.0	(d4) $PGF_{2\alpha}$.IS	1.5803
5-oxoETE	317.0 / 203.0	(d7) 5-oxoETE.IS	0.8498
5,15 diHETE	335.0 / 201.0	(d4) LTB_4.IS	0.5267
5,6 diHETE	335.0 / 115.0	(d4) LTB_4.IS	0.4905
5,6 DiHETrE	337.0 / 145.0	(d11) 11,12 DiHETrE.IS	3.6065
5,6 EpETrE	319.0 / 191.0	(d11) 11,12 DiHETrE.IS	3.7275
6,15-dk-,dh-$PGF_{1\alpha}$	369.0 / 267.0	(d4) $PGF_{2\alpha}$.IS	0.4899
6k PGE_1	367.0 / 331.0	(d4) PGE_2.IS	0.9207
6k $PGF_{1\alpha}$	369.0 / 245.0	(d4) 6k $PGF_{1\alpha}$.IS	3.6495
6R-LXA_4	351.0 / 217.0	(d4) LTB_4.IS	0.1535
6S-LXA_4	351.0 / 115.0	(d4) LTB_4.IS	0.2920
6t LTB_4	335.0 / 195.0	(d4) LTB_4.IS	0.9015
6t, 12epi LTB_4	335.0 / 195.0	(d4) LTB_4.IS	0.6674
7-HDoHE	343.0 / 141.0	(d8) 5-HETE.IS	0.5523
7R Maresin-1	359.0 / 177.0	(d4) LTB_4.IS	0.2837
8-HDoHE	343.0 / 109.0	(d8) 5-HETE.IS	0.4476
8-HEPE	317.0 / 155.0	(d8) 5-HETE.IS	1.1697
8-HETE	319.0 / 155.0	(d8) 5-HETE.IS	2.3110
8-HETrE	321.0 / 157.0	(d8) 5-HETE.IS	1.8141
8-iso 15k $PGF_{2\beta}$	351.0 / 219.0	(d4) $PGF_{2\alpha}$.IS	3.6218
8-iso $PGF_{2\alpha}$III	353.0 / 193.0	(d4) $PGF_{2\alpha}$.IS	1.9211
8-iso $PGF_{3\alpha}$	351.0 / 307.0	(d4) $PGF_{2\alpha}$.IS	--
8,15 diHETE	335.0 / 235.0	(d4) LTB_4.IS	0.1268
8,9 DiHETrE	337.0 / 127.0	(d11) 8,9 DiHETrE.IS	5.5366
8,9 EpETrE	319.0 / 155.0	(d11) 8,9 DiHETrE.IS	1.2994
9 oxoOTrE	291.0 / 185.0	(d7) 5-oxoETE.IS	2.2547
9-HEPE	317.0 / 149.0	(d8) 5-HETE.IS	0.6765
9-HETE	319.0 / 123.0	(d8) 5-HETE.IS	0.2492
9-HODE	295.0 / 171.0	(d4) 9-HODE.IS	1.4704
9-HOTrE	293.0 / 171.0	(d4) 9-HODE.IS	2.0060
9-Notrooleate	326.0 / 168.0	(d5) EPA.IS	--
9-oxoODE	293.0 / 185.0	(d7) 5-oxoETE.IS	1.4353
9,10 diHODE	311.0 / 201.0	(d4) 9,10 diHOME.IS	--
9,10 diHOME	313.0 / 201.0	(d4) 9,10 diHOME.IS	16.4260
9,10 EpODE	293.0 / 171.0	(d4) 9,10 diHOME.IS	--
9,10 EpOME	295.0 / 171.0	(d4) 9,10 diHOME.IS	3.7629
9,10,13 triHOME	329.0 / 171.0	(d4) 9-HODE.IS	3.1287
9,12,13 triHOME	329.0 / 211.0	(d4) 9-HODE.IS	6.1871
ADA	331.0 / 287.0	(d5) EPA.IS	--
ARA	303.0 / 259.0	(d5) EPA.IS	0.3304
bicyclo PGE_2	333.0 / 175.0	(d4) PGE_2.IS	0.7943

d17 6k $PGF_{1\alpha}$	367.0 / 163.0	(d4) $PGF_{2\alpha}$.IS	2.0835
dh $PGF_{2\alpha}$	355.0 / 283.0	(d4) $PGF_{2\alpha}$.IS	--
DHA	327.0 / 283.0	(d5) EPA.IS	0.7247
dhk PGD_2	351.0 / 207.0	(d4) PGD_2.IS	2.3400
dhk PGE_2	351.0 / 207.0	(d4) PGE_2.IS	0.5290
dhk $PGF_{2\alpha}$	353.0 / 291.0	(d4) dhk $PGF_{2\alpha}$.IS	4.9299
dihomo 15d PGD_2	361.0 / 299.0	(d4) 15d PGJ_2.IS	--
dihomo PGD_2	379.0 / 299.0	(d4) PGD_2.IS	--
dihomo PGE_2	379.0 / 299.0	(d4) PGE_2.IS	--
dihomo $PGF_{2\alpha}$	381.0 / 337.0	(d4) $PGF_{2\alpha}$.IS	2.8120
dihomo PGJ_2	361.0 / 299.0	(d4) 15d PGJ_2.IS	--
EPA	301.0 / 257.0	(d5) EPA.IS	1.9254
HXA_3	335.0 / 195.0	(d4) LTB_4.IS	--
HXB_3	335.0 / 183.0	(d4) LTB_4.IS	--
LTB_4	335.0 / 195.0	(d4) LTB_4.IS	1.2507
LTC_4	624.0 / 272.0	(d4) LTB_4.IS	0.3364
LTD_4	495.0 / 177.0	(d4) LTB_4.IS	1.0476
LTE_4	438.0 / 333.0	(d4) LTB_4.IS	0.9368
LXA_5	349.0 / 215.0	(d4) LTB_4.IS	0.3762
LXB_4	351.0 / 221.0	(d4) LTB_4.IS	0.3733
PD1	359.0 / 153.0	(d4) LTB_4.IS	--
PGA_2	333.0 / 271.0	(d4) 15d PGJ_2.IS	11.4540
PGB_2	333.0 / 271.0	(d4) 15d PGJ_2.IS	1.0891
PGD_1	353.0 / 235.0	(d4) PGD_2.IS	1.0006
PGD_2	351.0 / 271.0	(d4) PGD_2.IS	1.6144
PGD_3	349.0 / 269.0	(d4) PGD_2.IS	0.5356
PGE_1	353.0 / 235.0	(d4) PGE_2.IS	1.4269
PGE_2	351.0 / 271.0	(d4) PGE_2.IS	7.8596
PGE_3	349.0 / 269.0	(d4) PGE_2.IS	1.3408
$PGF_{1\alpha}$	355.0 / 293.0	(d4) $PGF_{2\alpha}$.IS	3.0675
$PGF_{2\alpha}$	353.0 / 193.0	(d4) $PGF_{2\alpha}$.IS	3.4181
$PGF_{3\alpha}$	351.0 / 193.0	(d4) $PGF_{2\alpha}$.IS	1.2284
PGJ_2	333.0 / 189.0	(d4) 15d PGJ_2.IS	3.6267
PGK_1	351.0 / 251.0	(d4) PGD_2.IS	4.2079
PGK_2	349.0 / 249.0	(d4) PGE_2.IS	1.8211
RvD1	375.0 / 141.0	(d4) LTB_4.IS	0.5531
RvD2	375.0 / 175.0	(d4) LTB_4.IS	0.3639
RvD5	359.2 / 199.0	(d4) LTB_4.IS	--
RvE1	349.0 / 195.0	(d4) LTB_4.IS	0.2271
tetranor 12-HETE	265.0 / 109.0	(d8) 15-HETE.IS	2.0436
tetranor-PGDM	327.0 / 247.0	(d4) PGD_2.IS	0.0984
tetranor-PGEM	327.0 / 291.0	(d4) PGE_2.IS	0.4032

tetranor-PGFM	329.0 / 247.0	(d4) $PGF_{2\alpha}$.IS	--
TXB_1	371.0 / 171.0	(d4) TXB_2.IS	3.6782
TXB_2	369.0 / 169.0	(d4) TXB_2.IS	6.3658
TXB_3	367.0 / 169.0	(d4) TXB_2.IS	5.4993

4.6 Protocol 5 – Dietary Oil Oxylipin Protocol

Oxylipins from dietary oil capsules were extracted, analyzed by HPLC-MS/MS on a Luna 5µm C18(2) 100Å 250 x 2.0 mm column, and quantified. Oil capsules were punctured using a surgical blade and oil was removed and transferred to a prepared 2.0 mL vial. To prepare for oxylipin analysis, 1350 µL of 100:1:2 Methanol:Formic Acid:antioxidant cocktail (0.2 mg/mL BHT, 0.2 mg/mL EDTA, 2.0 mg/mL TPP, 2.0 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:water) was added to 0.02 g of oil. Samples were vortexed and 750 µL was added to 3750 µL water at pH3, and 130 µL of deuterated internal standard (composition listed in **Table 0-1**). Samples were then acidified to pH3.

Solid phase extraction was prepared for by first setting up a Strata-X SPE (Phenomenex) (33 u, 60 mg/3mL) column for each sample. Each column was pre-conditioned with 3.5 mL 100% methanol and 3.5 mL water at pH3. Sample was applied to column and allowed to drip through by gravity. Meanwhile, 1.0 ml of 10% methanol in water at pH3 was added to the sample vial for rinse. This was then vortexed, centrifuged at 4°C, 3000 rpm for 5 minutes, and applied to column. The column was then rinsed with 2.0 mL pH3 and 1.0 mL hexane and pushed through to dry. Sample was then eluted into new 1.5 ml microfuge tubes with 1.0 mL 100% methanol. Samples were stored at -80^{0}C until analysis on HPLC-MS/MS.

When ready to run on HPLC, samples were dried down in a nitrogen evaporator bath at 37^{0}C and reconstituted in 1.3 mL Solvent A (water-acetonitrile-formic acid [70:30:0.02 v/v/v]). Samples were then vortexed and centrifuged at 14000 g for 10 minutes at 4^{0}C then transferred into labeled GC vials. Samples were run on a Shimadzu Nexera XR HPLC and ABSciex QTRAP 6500 MS with triple quadrapole electrospray ionization (IonDrive Turbo V). Samples were analyzed using MultiQuant Version 3.0. The analyte peak areas were divided by surrogate

internal standard, normalized to the detector response factor, and converted to M. The dose response factors are provided in **Table 0-2**.

4.7 Additional Results

Table 0-4 Analyzed capsule composition of investigational oils as a percentage per capsule. *Average percent composition of fatty acids present in one 1g oil capsule.*

ALA	
Fatty Acid	**Capsule Composition (%)**
C16:0	5.4
C18:0	2.9
C18:1ω7 (cis)	16
C18:2ω6	15
C18:3ω3	60
C12:0	<1%
C14:0	<1%
C15:0	<1%
C16:1 (trans)	<1%
C18:1ω7 (cis)	<1%
C20:0	<1%
C20:1ω9	<1%
C20:2	<1%
C20:3ω3	<1%
C22:0	<1%
DHA	
Fatty Acid	**Capsule Composition (%)**
C16:0	2.9
C18:0	4.4
C18:1	7.8
C18:1ω7 (cis)	1.4
C18:2ω6	1.1
C20:1	2.1
C20:4ω6	2.9
C20:5	11
C22:5ω6	3.7
C22:5ω3	2.3
C22:6ω3	54
C14:0	<1%
C15:0	<1%
C16:1(trans)	<1%
C16:1	<1%
C18:3ω6	<1%
C18:3ω3	<1%
C20:0	<1%

C20:2	<1%
C20:3ω6	<1%
C20:3ω3	<1%
C22:0	<1%
C22:1	<1%
C22:2	<1%
C22:4ω6	<1%
C24:0	<1%
C24:1	<1%

Table 0-5. Plasma fatty acids for individuals consuming ALA and DHA oil treatments (umol/L).
Data represents sexes combined. (*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between treatments for the same time point ($p < 0.05$). Values represent the mean±SEM.

	ALA						DHA					
Fatty Acid	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28
C12:0	9.6 ± 2.6	8.4 ± 1.9	6.0 ± 1.5	9.4 ± 2.0	10 ± 2.8	9.7 ± 2.8	13 ± 3.2	7.3 ± 1.6	5.0 ± 0.9*	9.7 ± 2.5	4.3 ± 0.8*	10 ± 2.7
C14:0	88 ± 14	87 ± 10	69 ± 12	104 ± 13	92 ± 14	96 ± 14	107 ± 13	94 ± 16	74 ± 12*	84 ± 11	95 ± 24	85 ± 17
C14:1ω9	5.2 ± 1.6	5.3 ± 1.2	3.1 ± 1.2	6.6 ± 2.0†	5.9 ± 1.4	5.1 ± 1.6	7.1 ± 1.4	5.7 ± 1.7	3.0 ± 0.9*	3.7 ± 1.0*	4.3 ± 2.7	5.2 ± 1.8
C16:0	2268±196	2287±155	2010±135	2690± 213*†	2346 ±167	2567 ± 176†	2440±203	2228±192	2057±125	2278 ±188	2212±158	2261 ± 232
C16:1ω7 (trans)[2]	36 ± 4.3	39 ± 3.2	28 ± 3.1	45 ± 4.4*†	38 ± 4.5	41 ± 5.2	42 ± 4.1	41 ± 4.2	32 ± 2.9*	36 ± 3.2	38 ± 3.6	39 ± 4.1
C16:1ω7[1,2,3]	181 ± 31	180 ± 30	140 ± 24	231 ±38*†	179 ± 27†	205 ± 32†	195 ± 33	167 ± 28	132 ± 19*	159 ± 29	116 ± 14*	151 ± 30*
C17:0[1,2]	22 ± 1.2	24 ± 1.0	21 ± 1.3	26 ± 2.1*	24 ± 1.9	28 ± 2.2*	25 ± 1.3	23 ± 1.2	23 ± 1.0	25 ± 1.5	25 ± 1.8	26 ± 1.7
C18:0[2]	681 ± 40	682 ± 39	621 ± 45	781 ± 50*	679 ± 29	798 ± 46*	752 ± 39	680 ± 36	647 ± 35	729 ± 57	683 ± 45	709 ± 56
C18:1ω9[2,3]	1762 ± 154	1854±107	1514±92	2219±236*†	1770 ± 147	2072±208*†	1934±142	1642±135	1477±97*	1664 ± 141	1506±141*	1623±147*
C18:1ω7(cis)	125 ± 12†	141 ± 14	127 ± 14	152 ± 11*†	135 ± 11†	153 ± 17*†	148 ± 12	136 ± 11	130 ± 11	127 ± 8.0	106 ± 8.4*	126 ± 13
C18:2ω6[2,3]	2494± 126†	2702±146	2460±148	3080±173*†	2606 ±110	3034±166*†	2918±209	2654 ±199	2396±144*	2497± 164*†	2290±185*	2525±234*
C18:3ω6[2]	39 ± 5.5†	36 ± 3.8	26 ± 2.3	41 ± 4.9†	33 ± 3.8	40 ± 3.8†	48 ± 6.6	41 ± 5.9	29 ± 3.6*	31 ± 4.7*	27 ± 5.5*	28 ± 5.6*
C18:3ω3[2,3]	67 ± 11	111± 13*†	113± 14*†	138 ± 12*†	129 ± 13*†	146 ± 17*†	81 ± 6.8	66 ± 6.4	61 ± 8.9	65 ± 9.4	69 ± 11	63 ± 8.5
C20:0	24 ± 1.4†	25 ± 1.0	24 ± 1.2	28 ± 1.6	26 ± 1.3	29 ± 1.3*	28 ± 1.5	26 ± 1.9	24 ± 1.5*	25 ± 1.9	25 ± 2.1	27 ± 2.0
C20:1[2,3]	17 ± 1.3	18 ± 1.9	16 ± 2.1	20 ± 2.0	18 ± 2.0†	19 ± 2.0†	19 ± 1.7	16 ± 1.2	16 ± 1.6	17 ± 1.4	13 ± 1.4*	16 ± 1.3*
C20:2	20 ± 3.1	22 ± 3.4	20 ± 4.5	21 ± 2.0	21 ± 3.1	22 ± 2.1†	25 ± 2.8	20 ± 2.6	18 ± 2.3	22 ± 3.7	16 ± 2.4*	16 ± 2.0*
C20:3ω6[2,3]	150 ± 17	153 ± 18	131 ± 19	149 ± 14	143 ± 17†	156 ± 13†	172 ± 20	155 ± 19	128 ± 14*	130 ± 18*	108 ± 14*	108 ± 19*
C20:4ω6	691 ± 46	715 ± 46	650 ± 51	759 ± 55	677 ± 40	738 ± 37	744 ± 47	726 ± 44	730 ± 38	739 ± 38	654 ± 44*	704 ± 48
C20:3ω3	2.3 ± 1.6	3.4 ± 1.6	2.5 ± 1.3	4.3 ± 1.2	3.4 ± 1.4	4.8 ± 1.2	4.1 ± 3.0	1.0 ± 0.4	4.0 ± 3.1	1.0 ± 0.3	6.5 ± 5.4	0.5 ± 0.2
C20:5ω3[2,3]	81 ± 15	73 ±8.8†	68 ±7.4†	101 ± 10†	93 ± 8.4†	120 ±17*†	86 ± 13	143 ± 17*	178 ± 14*	211 ± 18*	218 ± 24*	267 ± 25*

C22:0	60 ± 4.5	63 ± 2.8	60 ± 3.3	71 ± 5.2*†	64 ± 3.9	73 ± 4.8*	68 ± 3.9	65 ± 6.7	58 ± 4.7	59 ± 5.1	59 ± 5.6	66 ± 6.2
C22:1ω9	2.2 ± 0.4	2.4 ± 0.3	2.3 ± 0.2	2.8 ± 0.2†	2.4 ± 0.3	2.8 ± 0.4	2.8 ± 0.3	2.3 ± 0.3	2.2 ± 0.3	2.0 ± 0.3*	2.1 ± 0.2	2.4 ± 0.3
C22:2	2.1 ± 0.3	2.6 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.3	2.7 ± 0.2	2.4 ± 0.2	2.3 ± 0.3	3.1 ± 1.1	2.3 ± 0.3	1.6 ± 1.1	2.1 ± 0.3
C22:4ω6[2,3]	20 ± 1.7	20 ± 1.6	18 ± 1.6	22 ± 2.1†	19 ± 1.3†	20 ± 1.7†	21 ± 1.5	19 ± 1.3	16 ± 0.9*	15 ± 1.7*	12 ± 2.0*	12 ± 2.1*
C22:5ω6[3]	13 ± 1.9	13 ±1.9†	12 ±2.2†	12 ± 2.2†	11 ± 1.9†	12 ± 1.5†	14 ± 1.8	19 ± 2.3*	18 ± 1.9*	19 ± 1.9*	17 ± 1.8	19 ± 1.4*
C22:5ω3[2,3]	39 ± 3.4	40 ± 2.9	37 ± 2.4	51 ± 5.1*†	45 ± 3.7†	57 ± 5.1*†	45 ± 2.6	43 ± 3.0	39 ± 2.2	38 ± 3.4	36 ± 2.7*	40 ± 3.7
C22:6ω3[2,3]	169 ± 14	175±15†	159±16†	193 ± 16†	176 ± 17†	192 ± 15†	156 ± 15	240 ± 24*	312 ± 27*	400 ± 32*	406 ± 29*	456 ± 36*
C24:0	51 ± 3.6	54 ± 2.6	51 ± 2.9	59 ± 2.7	56 ± 3.4	64 ± 3.6*†	58 ± 3.9	54 ± 4.8	49 ± 4.4	52 ± 4.7	50 ± 5.3*	52 ± 4.3
C24:1ω9[1]	89 ± 7.7	97 ± 6.1	95 ± 6.0	101 ± 5.5	98 ± 5.9	107 ± 5.2*	101 ± 6.0	95 ± 7.5	92 ± 8.6	96 ± 7.2	93 ± 10	101 ± 9.0
Total Saturated	3204±251	3231±198	2864±181	3772±271*†	3297 ±205	3665± 232*†	3492± 254	3182 ±248	2941± 165*	3261 ±255	3161 ± 228	3240 ± 313
Total Monounsaturated[2,3]	2217±194	2337±154	1924±134	2421 ± 166†	2244± 176†	2440 ± 212†	2448±189	2105± 177*	1884±122*	2104 ± 172*	1878 ± 62*	2064±188*
Total Omega-6[3]	3426±188	3661±201	3315±212	4084±236*†	3510 ±161	4022±201*†	3733±190	3634 ±258	3335±189*	3454 ± 215*	3124± 229*	3411± 296*
Total Omega-3[2,3]	358 ± 30	402 ± 29	380 ± 31†	487 ± 33*†	446 ±32*†	518 ± 33*†	372 ± 30	494 ± 44*	593 ± 41*	715 ± 44*	736 ± 52*	826 ± 49*
Total Polyunsaturated[2]	3787±202	4066±216	3697±236	4574 ±256*	3959 ±179	4543±208*†	4097±199	4130 ±282	3931 ±216	4170 ± 229	3867 ± 252	3975 ± 191

[1]Sex Effect; [2]Time Effect; [3]Treatment Effect

Table 0-6. Plasma fatty acids for males and females consuming ALA oil treatment (umol/L).
(*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Fatty Acid	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
C12:0	7.3 ± 2.5	12 ± 4.7	4.7 ± 1.5	12 ± 2.8	3.7 ± 0.9	8.3 ± 2.5	5.7 ± 1.3	13 ± 3.1	7.6 ± 1.8	13 ± 5.3	8.1 ± 2.3	11 ± 5.3
C14:0	801 ± 21	96 ± 19	65 ± 13	109 ± 9.6	54 ± 9.7	85 ± 21	90 ± 20	118 ± 17	87 ± 19	98 ± 21	93 ± 25	99 ± 15
C14:1ω9	3.9 ± 2.2	6.4 ± 2.4	2.8 ± 1.4	7.7 ± 1.4	2.5 ± 1.0	3.6 ± 2.2	4.2 ± 1.7	9.0 ± 3.5	5.4 ± 2.3	6.4 ± 1.9	4.3 ± 2.6	5.8 ± 1.9
C16:0	1997± 213	2540± 307	1927± 127	2647± 194	1755 ± 75	2266± 209	2418 ± 337	2963± 237	2232± 171	2460± 297	2455± 246*	2679± 267
C16:1ω7 (trans)[2]	29 ± 5.1	42 ± 6.2	32 ± 3.4	46 ± 3.6	25 ± 3.9	31 ± 4.9	42 ± 6.9*	48 ± 5.9	36 ± 5.8	39 ± 7.5	42 ± 6.8*	41 ± 8.7
C16:1ω7[1,2,3]	119 ± 24	244 ± 46	118 ± 18	242 ± 47	92 ± 18	189 ± 33	151 ± 38	311 ± 50*	133 ± 25	224 ± 42	147 ± 30	262 ± 48
C17:0[1,2]	23 ± 2.1	21 ± 1.4	22 ± 1.2	25 ± 1.5	21 ± 1.1	22 ± 2.4	27 ± 3.8	24 ± 2.0	28 ± 2.5*	20 ± 1.7	29 ± 3.6*	27 ± 2.8*
C18:0[2]	625 ± 50	737 ± 59	596 ± 38	769 ± 47	560 ± 28	682 ± 81	741 ± 78	822 ± 64	708 ± 43	650 ± 38	784 ± 76*	812 ± 58
C18:1ω9[2,3]	1713± 250	1811± 201	1691± 137	2017± 147	1354 ± 81	1674± 137	2251± 419*	2186± 262	1935± 261	1604± 128	2057 ± 258	2086± 351
C18:1ω7(cis)	111 ± 14	139 ± 19	111 ± 9.5	171 ± 22*	103 ± 15	151 ± 19	138 ± 16	166 ± 13	132 ± 11	137 ± 21	142 ± 23	164 ± 26
C18:2ω6[2,3]	2333± 126	2655± 209	2330 ± 83	3074± 177	2239 ± 82	2680± 261	3069± 251*	3091± 261	2723± 112	2490± 188	3226±207*†	2841± 251
C18:3ω6[2]	40 ± 11	38 ± 4.1	35 ± 7.3	37 ± 3.0	21 ± 1.8*	30 ± 3.3	38 ± 7.7	43 ± 6.6	36 ± 6.4	30 ± 4.2	40 ± 4.5	39 ± 6.6
C18:3ω3[2,3]	73 ± 20	61 ± 9.3	93 ± 13	130 ± 22*	99 ± 15	128 ± 22*	140 ± 21*	136 ± 14*	137 ± 24*	121 ± 12*	150 ± 25*	142 ± 25*
C20:0	23 ± 2.6	26 ± 1.1	24 ± 1.3	27 ± 1.1	23 ± 0.7	26 ± 2.2	28 ± 3.0*	28 ± 1.5	27 ± 2.2	25 ± 1.3	30 ± 2.6*	29 ± 0.9
C20:1[2,3]	16 ± 2.1	18 ± 1.7	15 ± 1.0	20 ± 3.4	13 ± 1.0	18 ± 4.0	20 ± 3.4	19 ± 2.3	17 ± 2.9	18 ± 2.9	17 ± 1.4	22 ± 3.6
C20:2	14 ± 1.4	25 ± 5.5	17 ± 3.8	26 ± 5.4	13 ± 1.1	26 ± 8.3	19 ± 2.7	23 ± 2.8	20 ± 4.8	22 ± 4.3	22 ± 3.5	23 ± 2.6
C20:3ω6[2,3]	121 ± 10	178 ± 29	123 ± 12	183 ± 32	96 ± 8.9	166 ± 31	128 ± 19	170 ± 17	130 ± 16	156 ± 30	139 ± 16	173 ± 17
C20:4ω6	648 ± 63	735 ± 66	636 ± 55	794 ± 61	570 ± 57	730 ± 73	720 ± 103	798 ± 47	684 ± 67	669 ± 52	742 ± 74	734 ± 26
C20:3ω3	0.6 ± 0.2	4.1 ± 3.1	1.5 ± 0.5	5.3 ± 3.1	1.0 ± 0.3	4.0 ± 2.6	2.6 ± 0.8	5.9 ± 2.2	1.8 ± 0.5	5.0 ± 2.7	5.7 ± 1.9	3.9 ± 1.5
C20:5ω3[2,3]	91 ± 29	71 ± 9.1	63 ± 12	83 ± 12	59 ± 13	78 ± 6.2	86 ± 14	116 ± 13*	90 ± 14	95 ± 10	98 ± 17†	141 ± 27*
C22:0	58 ± 8.9	62 ± 2.8	60 ± 4.2	66 ± 3.8	58 ± 3.3	62 ± 6.1	73 ± 9.5	70 ± 5.4	68 ± 5.7	59 ± 5.2	77 ± 8.6	70 ± 4.7
C22:1ω9	1.8 ± 0.5	2.6 ± 0.6	2.3 ± 0.3	2.5 ± 0.5	2.2 ± 0.3	2.4 ± 0.4	2.7 ± 0.4	2.8 ± 0.2	2.6 ± 0.5	2.3 ± 0.3	3.3 ± 0.7*†	2.4 ± 0.4
C22:2	1.8 ± 0.5	2.4 ± 0.1	2.4 ± 0.3	2.9 ± 0.3	2.3 ± 0.3	2.6 ± 0.4	2.4 ± 0.4	2.4 ± 0.4	2.6 ± 0.3	2.2 ± 0.6	2.8 ± 0.3	2.5 ± 0.2
C22:4ω6[2,3]	19 ± 2.2	22 ± 2.7	18 ± 1.7	22 ± 2.5	15 ± 1.2	20 ± 2.5	21 ± 3.7	24 ± 2.3	18 ± 2.1	19 ± 1.8	20 ± 2.7	21 ± 2.4
C22:5ω6[3]	10 ± 2.2	16 ± 2.8	8.9 ± 1.6	16 ± 2.8	7.5 ± 1.6	16 ± 3.3	8.5 ± 3.2	16 ± 2.3	9.3 ± 2.4	13 ± 2.8	12 ± 2.4	13 ± 2.1
C22:5ω3[2,3]	39 ± 5.7	38 ± 4.2	41 ± 5.5	39 ± 2.5	34 ± 3.1	40 ± 3.4	53 ± 9.0*	48 ± 5.5	51 ± 6.1*	39 ± 3.4	58 ± 9.0*	55 ± 5.5*
C22:6ω3[2,3]	145 ± 17	194 ± 18	146 ± 16	204 ± 21	128 ± 17	190 ± 19	168 ± 23	218 ± 19	165 ± 22	187 ± 27	179 ± 22	204 ± 22
C24:0	49 ± 6.4	53 ± 4.0	51 ± 2.6	57 ± 4.5	50 ± 1.8	53 ± 5.6	59 ± 4.7	58 ± 3.0	57 ± 3.5	55 ± 6.2	65 ± 6.0*	62 ± 4.6
C24:1ω9[1]	76 ± 8.8	102 ± 11	84 ± 1.8	110 ± 9.4	83 ± 2.1	107 ± 9.4	93 ± 6.6	109 ± 7.9	93 ± 4.4	102 ± 11	104 ± 8.7*	111 ± 6.2
Total Saturated	2862± 294	3546± 379	2750± 173	3712± 224	2524± 93	3204± 284	3449± 453	4094± 278	3213± 239	3380± 355	3542± 361*	3789± 318
Total Monounsaturated[2,3]	2071± 293	2365± 268	2056± 162	2617± 216	1674± 114	2175± 191	2290± 308	2553± 142	2355± 303	2133± 199	2516± 319*	2350± 302
Total Omega-6[3]	3185± 204	3668± 300	3168± 130	4153± 253	2962 ± 128	3669 ± 350	4003 ± 378*	4165 ± 317	3620 ± 186	3399 ± 273	4201 ± 288*	3844 ± 287
Total Omega-3[2,3]	349 ± 57	367 ± 25	345 ± 41	460 ± 26	320 ± 41	439 ± 31	450 ± 58	524 ± 30*	445 ± 54	448 ± 39	491 ± 50*	546 ± 45*
Total Polyunsaturated[2]	3536± 241	4038± 310	3515± 148	4616± 250	3284 ± 159	4111 ± 373	4456 ± 425*	4692 ± 319	4068± 221	3849± 294	4695± 317*	4392± 284

[1]Overall Sex Effect; [2] Overall Time Effect; [3]Overall Treatment Effect

Table 0-7. Plasma fatty acids for males and females consuming DHA oil treatment (umol/L).
(*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Fatty Acid	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
C12:0	17 ± 5.8	10 ± 2.5	8.6 ± 3.5	6.3 ± 0.9	4.1± 1.7*	5.9 ± 0.5	11 ± 5.4	8.8 ± 1.9	4.8 ± 1.6	4.0 ± 0.9	7.6± 4.0*	12 ± 3.8
C14:0	115 ± 21	99 ± 17	102 ± 31	85 ± 13	74 ± 22*	74 ± 4.7	83 ± 15*	86 ± 18	119 ± 54	74 ± 6.9	74 ± 30*	93 ± 20
C14:1ω9	6.4 ± 2.1	7.7 ± 2.0	5.8 ± 2.7	5.5 ± 2.2	3.4 ± 1.7	2.6 ± 0.3*	2.7 ± 1.1	4.5 ± 1.6	7.6 ± 5.9†	1.6 ± 0.3*	4.5 ± 3.5	5.8 ± 1.8
C16:0	2183± 158	2697± 360	1994± 167	2462± 335	1932± 148	2208 ± 205	2167± 134	2370 ± 337	2245 ± 292	2183± 185*	2122± 267	2376 ± 380
C16:1ω7(trans)[2]	41 ± 4.8	44 ± 7.1	38 ± 4.8	44 ± 7.2	33 ± 2.7	31 ± 5.8	41 ± 3.4	32 ± 4.6*	43 ± 6.0	33 ± 3.7	39 ± 4.0	40 ± 7.2
C16:1ω7[1,2,3]	122 ± 23	267 ± 48	110 ± 20	223 ± 44	98 ± 17	172 ± 27*	101 ± 9.7	207 ± 44*	109 ± 30†	122 ± 8.8*	106 ± 37	188 ± 41*
C17:0[1,2]	26 ± 1.2	23 ± 2.1	24 ± 1.4	22 ± 2.1	24 ± 1.3	22 ± 1.5	28 ± 1.6	22 ± 1.9	30 ± 2.2	22 ± 1.6	27 ± 2.0	25 ± 2.9
C18:0[2]	733 ± 42	771 ± 69	636 ± 45	725 ± 54	617 ± 58	683 ± 36	740 ± 57	720 ± 99	711 ± 88	661 ± 45	696 ± 41	720 ± 103
C18:1ω9[2,3]	1843± 170	2024± 239	1566± 155	1718± 231	1495± 140	1454± 148*	1711± 172	1624 ± 228	1789± 254†	1270 ± 76*	1685± 209	1571± 220*
C18:1ω7(cis)	135 ± 9.4	161 ± 23	118 ± 11	153 ± 18	120 ± 9.5	143 ± 20	122 ± 7.0	131 ± 14	126 ± 9.2†	90 ± 9.4*	107 ± 12	142 ± 21
C18:2ω6[2,3]	2798± 164	3038± 399	2492± 135	2815± 381	2430± 199	2355± 231*	2682± 193	2343± 252*	2476 ± 277	2136± 251*	2542± 173	2510± 425*
C18:3ω6[2]	43 ± 6.7	53 ± 12	37 ± 6.4	45 ± 10	29 ± 6.0*	28 ± 4.0*	30 ± 5.6*	32 ± 7.7*	35 ± 11†	20 ± 2.3*	29 ± 11*	27 ± 5.4*
C18:3ω3[2,3]	87 ± 11	75 ± 8.2	71 ± 11	62 ± 7.4	67 ± 16	53 ± 4.5	81 ± 18	51 ± 6.6	87 ± 22	54 ± 7.6	76 ± 16	52 ± 5.7
C20:0	27 ± 1.3	30 ± 2.6	23 ± 2.4	29 ± 2.4	24 ± 1.2	24 ± 3.1	26 ± 3.9	25 ± 1.8	26 ± 2.8	25 ± 3.4	25 ± 2.4	29 ± 3.1
C20:1[2,3]	18 ± 1.9	20 ± 3.0	15 ± 0.7	18 ± 2.2	15 ± 1.5	17 ± 3.2	17 ± 1.7	17 ± 2.3	12 ± 1.7*	13 ± 2.2*	16 ± 1.0	16 ± 2.3
C20:2	22 ± 3.6	27 ± 4.4	15 ± 0.7	25 ± 4.5	17 ± 3.8	19 ± 2.6	24 ± 5.8	21 ± 5.1	17 ± 5.4	16 ± 1.5*	13 ± 1.0	18 ± 3.5*
C20:3ω6[2,3]	141 ± 17	202 ± 33	119 ± 12	190 ± 30	115 ± 18	143 ± 22*	119 ± 22	139 ± 29*	106 ± 27†	110 ± 15*	86 ± 21*	127 ± 30*
C20:4ω6	682 ± 30	806 ± 85	639 ± 30	813 ± 67	708 ± 55	757 ± 54	748 ± 45	731 ± 62	684 ± 72	629 ± 58*	656 ± 35	745 ± 83
C20:3ω3	7.3 ± 5.9	0.9 ± 0.4	0.6 ± 0.4	1.4 ± 0.6	0.5± 0.3†	8.1 ± 6.6	0.9 ± 0.3	1.1 ± 0.4	1.1 ± 0.5†	11 ± 9.9*	0.7 ± 0.3	0.3 ± 0.2
C20:5ω3[2,3]	75 ± 17	97 ± 19	123± 18*	164± 27*	164± 16*	195 ± 23*	215± 25*	208 ± 28*	230 ± 20*	208 ± 43*	234± 38*	295 ± 31*
C22:0	66 ± 3.3	70 ± 7.4	57 ± 6	74 ± 12	58 ± 5.4	57 ± 8.9	59 ± 8.9*	59 ± 6.5	67 ± 5.6	53 ± 8.8*	61 ± 8.6*	70 ± 9.2
C22:1ω9	2.6 ± 0.4	3.0 ± 0.4	2.0 ± 0.3	2.7 ± 0.5	1.7 ± 0.3	2.8 ± 0.5	1.7 ± 0.3	2.3 ± 0.4	2.2 ± 0.3	2.0 ± 0.3*	2.4 ± 0.2	2.4 ± 0.5
C22:2	2.1 ± 0.3	2.8 ± 0.3	1.8 ± 0.4	2.8 ± 0.2	3.9 ±1.9*†	2.3 ± 0.6	1.9 ± 0.5	2.5 ± 0.3	1.8 ± 0.4	1.5 ± 0.6	1.6 ± 0.5	2.5 ± 0.3
C22:4ω6[2,3]	20 ± 1.0	23 ± 2.8	18 ± 1.0	21 ± 2.4	15 ± 1.1	18 ± 1.3*	14 ± 1.8*	16 ± 2.9*	10 ± 3.8*	13 ± 2.1*	11 ± 3.8*	13 ± 2.4*
C22:5ω6[3]	11 ± 1.9	16 ± 3.0	15 ± 2.2	22 ± 3.6*	17 ± 2.9*	21 ± 2.2*	19 ± 2.7*	19 ± 2.9	19 ± 3.8*†	15 ± 0.9	16 ± 1.3*	20 ± 2.1
C22:5ω3[2,3]	45 ± 4.0	44 ± 3.7	44 ± 3.7	43 ± 5.1	39 ± 3.4	38 ± 3.2	39 ± 4.8	38 ± 5.2	40 ± 5.3	33 ± 2.0*	38 ± 6.9	41 ± 4.1
C22:6ω3[2,3]	143 ± 22	170 ± 22	214± 28*	266± 38*	289± 43*	339 ± 32*	419± 51*	384 ± 43*	443± 52*†	376 ± 29*	389± 43*†	513 ± 45*
C24:0	55 ± 2.6	61 ± 7.5	48 ± 5.2	59 ± 7.8	49 ± 5.0	49 ± 8.3	51 ± 7.3	52 ± 6.7	53 ± 5.5	48 ± 8.9*	50 ± 6.5	54 ± 6.2
C24:1ω9[1]	91 ± 7.1	110 ± 8.5	80 ± 10	110 ± 7.3	86 ± 10	100 ± 15	93 ± 15	98 ± 6.5	87 ± 8.3	97 ± 19	83 ± 9.2	116 ± 12
Total Saturated	3223±215	3761± 457	2901± 254	3463± 419	2789± 221	3124 ± 247	3165± 222	3342 ± 450	3270 ± 448	3070± 232*	3062± 351	3389± 517
Total Monounsaturated[2,3]	2259±197	2636± 322	1935± 182	2275± 306	1852± 160	1923±207*	2089± 184	2116±294*	2176±302†	1630± 89*†	2044± 248	2081±297*
Total Omega-6[3]	3717±197	3752± 374	3335± 161	3932± 482	3331± 262	3340 ± 305	3636± 230	3301 ± 351	3346 ± 341	2939± 316*	3353± 186	3459 ± 545
Total Omega-3[2,3]	358 ± 44	387 ± 45	452 ± 51	536± 71*	560± 61*	633 ± 57*	755± 61*	681 ± 64*	801 ± 73*	681 ± 70*	737± 62*	901 ± 62*
Total Polyunsaturated[2]	4077± 204	4122± 394	3789± 163	4470± 526	3894± 320	3976 ± 319	4394± 246	3985 ± 369	4150 ± 373	3632 ± 342	4091± 180	3859 ± 353

[1]Overall Sex Effect; [2] Overall Time Effect; [3]Overall Treatment Effect

Table 0-8. Plasma oxylipins for individuals consuming ALA and DHA oil treatment grouped by parent PUFA (pM).
Data represents sexes combined. (*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between treatments for the same time point ($p < 0.05$). Values represent the mean±SEM.

	ALA						DHA					
	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28
LA Derived Oxylipins												
Total	28566±2663	31303±3994	31279±4405	41169±8681	27278±3241	29614±3976†	38894±7078	39508±11545	29653±4113	45016±9756	31967±6740	67803±17426*
LOX	27606±2659	30286±3879	30437±4390	40087±8577	26454±3129	28697±3869†	37969±7027	38657±11551	28641±4039	43947±9721	31167±6724	66776±17416*
cP450[1]	960±112	1017±159	841±94	1082±159	824±132	918±153	926±145	851±145	1012±191	1068±121	800±151	1027±204
DGLA Derived Oxylipins												
Total	391±59	335±74	313±50	375±58	312±66	364±56	362±42	378±63	386±61	404±51	391±69	442±66
LOX	163±11	152±14	154±11	201±22	151±14	193±19	184±24	175±25	186±18	195±16	155±14	189±20
COX	228±63	184±71	158±52	174±49	161±67	172±48	178±32	203±56	200±57	209±56	236±65	254±59
AA Derived Oxylipins												
Total	4079±257	3614±270	3689±256	4043±352	3980±509	4485±774	4436±597	3554±252	4070±342	4401±335	3726±241	4330±389
COX	734±92	500±55*	595±122	593±80	555±59	614±71	669±125	504±72	635±81	725±80	662±75	685±100
LOX	2471±203	2301±249	2185±187	2589±317	2666±502	2990±723	3032±523	2197±211	2522±272	2817±285	2310±215	2853±339
cP450	1344±88	1159±81*	1259±98	1275±40	1137±51*	1233±82	1228±70	1178±40	1330±93	1330±78	1146±59	1276±81
cP450 Epoxygenase	465±33	423±23	471±42	458±30	440±34	473±35	482±33	411±18	461±25	451±27	410±38	428±25
cP450 Hydroxylase	879±70	735±69	788±77	817±50	697±47*	760±68	746±42	766±44	868±72	879±64	737±42	849±80
Total Omega-6 Derived Oxylipins												
	33143±2732	35346±4229	35360±4217	45688±8918	31668±3395	34592±4399†	43831±7215	43548±11579	34210±4410	49935±9794	36171±6663	72686±17548*
ALA Derived Oxylipins												
Total	1049±152	1009±186	1006±156	1110±168	1207±150	1442±220	1038±189	971±163	1096±180	1145±149	925±179	1057±257
LOX	961±149	911±173	929±158	998±165	1060±134	1306±209	920±172	890±156	1006±182	1029±148	785±142	937±250
cP450	88±16	98±20	77±8.2	112±17	147±52	136±38	118±27	81±13	91±26	116±19	139±54	120±34
EPA Derived Oxylipins												
Total	335±55	345±49†	301±46†	400±86†	293±26†	373±58†	367±49	533±64*	684±83*	755±88*	745±75*	874±81*

LOX	253±41	270±42†	232±44†	303±74†	224±23†	292±52†	261±41	415±57*	524±62*	566±63*	542±62*	653±73*
DHA Derived Oxylipins												
Total[1]	2580±324	2466±303†	2323±328†	3108±618†	2247±240†	2662±333†	2360±267	4664±597*	5780±705*	6146±692*	6750±620*	7633±759*
LOX[1]	1403±230	1269±193†	1187±190†	1692±367†	1180±152†	1467±247†	1262±176	2898±469*	3680±605*	3725±449*	4137±422*	4927±593*
cP450[1]	1176±122	1197±138†	1136±146†	1416±271†	1067±108†	1195±128†	1098±113	1766±152*	2100±160*	2420±299*	2613±241*	2706±222*
cP450 Epoxygenase[1]	973±109	967±118†	923±122†	1120±201†	873±93†	1002±116†	925±98	1284±109*	1611±123*	1832±195*	1889±161*	1940±158*
Total Omega-3 Derived Oxylipins												
	3964±391	3820±387†	3630±412†	4618±732†	3748±371†	4477±498†	3765±415	6168±698*	7561±828*	8046±701*	8419±718*	9565±923*
Total Enzymatically Derived Oxylipins												
COX	1015±129	733±84*	793±112	828±97†	756±84*†	834±93†	888±139	850±93	990±93	1095±76	1066±90	1158±124*
LOX	32800±2934	35131±4273	35056±4253	45778±9060	31686±3427	34881±4485†	43583±7230	45165±11980	36475±4851	52202±10029	39030±6575	76258±17397*
cP450[1]	3651±258	3546±342	3382±254†	3982±392†	3245±270†	3563±342†	3474±307	3995±293	4693±380*	5123±405*	4902±389*	5350±454*
cP450 Epoxygenase	2486±191	2506±287	2312±215†	2771±301†	2284±234†	2528±303†	2450±267	2628±243	3175±276*	3468±247*	3238±301*	3515±369*
cP450 Hydroxylase[1]	1165±86	1040±87†	1069±96†	1210±112†	961±61†	1035±80†	1024±58	1366±113*	1518±132*	1656±191*	1664±121*	1835±187*
Total Oxylipins	37107±3015	39167±4510	38996±4228	50313±9460	35419±3676	39070±4761†	47596±7373	49717±12121	41773±5072	57984±10232	44593±6607	82252±17383*

[1]Overall Sex Effect; [2] Overall Time Effect; [3]Overall Treatment Effect

Table 0-9 Plasma oxylipins for individuals consuming ALA and DHA oil treatment grouped by parent PUFA (pM)
Data represents sexes combined. (*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between treatments for the same time point ($p < 0.05$). Values represent the mean±SEM.

	ALA						DHA					
Oxylipin	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28
LA - LOX Oxylipins												
9-HODE[2]	9055 ± 857	8683 ± 925	7783 ± 642	10557 ± 1557	8143 ± 969	8576 ± 985†	8695 ± 1416	8299 ± 1422	8947 ± 1304	9188 ± 1444	7447 ± 890	12683 ± 2427*
13-HODE[2]	4854 ± 573	4936 ± 529	3713 ± 251	5589 ± 685	4294 ± 431	4410 ± 501	4961 ± 683	4380 ± 663	4753 ± 668	4957 ± 652	3679 ± 365*	4940 ± 672
9-oxoODE	93 ± 15	95 ± 18	68 ± 22	130 ± 30	119 ± 21	113 ± 29	99 ± 25	159 ± 43	130 ± 24	144 ± 46	91 ± 27	166 ± 42
13-oxoODE[2]	477±135	656 ± 152	591 ± 104	925± 188*†	595 ± 95	644 ± 118	626 ± 184	692 ± 169	494 ± 123	553 ± 217	547 ± 115	497 ± 167
9,10,13 triHOME[4]	6026 ± 824	7963± 1398	8400 ± 2154	12619 ± 5562	5950 ± 894	6831 ± 1512†	8561 ± 1309	12536 ± 5960	6772 ± 1425	14107 ± 4321	9261 ± 3342	23718 ± 8398*
9,12,13 triHOME[4]	7036 ± 817	7955 ± 1498	9886 ± 2513	10775 ± 2788	7354 ± 1150	8122 ± 1829†	15037 ± 6294	12592 ± 5876	7548 ± 1582	15021 ± 4626	10144 ± 3295	26193 ± 8977
LA - cP450 Oxylipins												
9,10 diHOME[2]	276 ± 31	278 ± 38	257 ± 35	305 ± 56	243 ± 37	238 ± 40	299 ± 52	273 ± 59	265 ± 41	308 ± 53	223 ± 33	322 ± 69
9,10 EpOME[2]	66 ± 14	64 ± 16	78 ± 24	85 ± 20	65 ± 15	74 ± 19	52 ± 18	50 ± 10	69 ± 25	65 ± 19	57 ± 24	77 ± 20
12,13 diHOME[3]	446 ± 70	477 ± 84†	376 ± 47	473 ± 72	345 ± 52	377 ± 66	437 ± 85	350 ± 65	403 ± 78	469 ± 58	306 ± 45	397 ± 91
12,13 EpOME	173 ± 24	198 ± 44	128 ± 20†	214 ± 43	176 ± 68	230 ± 68	130 ± 20	172 ± 36	274 ± 69*	226 ± 43	215 ± 76	217 ± 63
GLA - LOX Oxylipins												
13-HOTrE-γ	106 ± 11	94 ± 16	80 ± 11	102 ± 14	98 ± 12	128 ± 16	139 ± 21	108 ± 24	99 ± 14	114 ± 17	87 ± 10*	111 ± 21
DGLA - COX Oxylipins												
$PGF_{1\alpha}$[4]	176 ± 39	113 ± 25	158 ± 52	174 ± 49	99 ± 29	162 ± 52†	178 ± 38	203 ± 66	205 ± 63	216 ± 61	178 ± 31	254 ± 59*
DGLA - LOX Oxylipins												
8-HETrE[2]	8.3±3.6†	31 ± 4.9*	17 ± 3.4	34 ± 9.1*	17 ± 4.8	28 ± 5.6*	25 ± 6.7	23 ± 6.4	26 ± 4.9	28 ± 7.5	16 ± 4.9	19 ± 10
15-HETrE[3]	153 ± 15	120±11*†	137 ± 12	165 ± 17	133 ± 14	164 ± 17	159 ± 18	153 ± 21	160 ± 18	164 ± 14	138 ± 12	169 ± 22
EDA - LOX Oxylipins												

15-oxoEDE	0.3 ± 02	1.4 ± 0.7	5.8 ± 2.1*	6.5± 4.6*†	3.6 ± 2.2	2.0 ± 1.3	0.4 ± 0.4	1.4 ± 0.8	2.5 ± 1.0	2.1 ± 1.1	2.1 ± 1.1	0.4 ± 0.4
AA - COX Oxylipins												
11βPGE$_2$[3,4]	24 ± 3.5	28 ± 2.4	20 ± 1.6	22 ± 2.1	24 ± 1.6	28 ± 3.5†	25 ± 3.2	23 ± 2.6	26 ± 2.3	27 ± 1.8	27 ± 1.9	39 ± 3.2*
12-HHTrE	108 ± 52	20 ± 10	137 ± 91	63 ± 30	60 ± 31	100 ± 69	48 ± 41	26 ± 17	67 ± 19	88 ± 39	113 ± 53	25 ± 17
6k PGF$_{1\alpha}$[3]	11 ± 2.8	6.6 ± 2.8	7.6 ± 2.3	6.3 ± 2.0	7.1 ± 1.6	9.5 ± 1.9	15 ± 4.1	11 ± 2.8	12 ± 1.7	8.8 ± 2.3	6.0 ± 1.6*	7.1 ± 2.5*
PGD$_2$[4]	17 ± 3.3	16 ± 3.1	6.7 ± 2.4	13 ± 4.4	13 ± 2.7†	16 ± 5.1†	19 ± 6.2	20 ± 6.8	13 ± 3.8	24 ± 4.7	30 ± 8.0	31 ± 6.8
PGE$_2$[3]	16 ± 1.6	18 ± 1.4	14 ± 0.6	16 ± 2.4	15 ± 1.2	18 ± 2.0	15 ± 2.2	16 ± 0.9	16 ± 1.2	19 ± 1.4	19 ± 1.2	22 ± 3.0*
PGF$_{2\alpha}$	50± 8.3†	24 ± 7.2*	20± 2.7*†	20 ± 3.6*	16 ± 2.0*	39 ± 7.3	17 ± 3.6	31 ± 6.5	38 ± 10*	34 ± 10	35 ± 11	31 ± 8.4
TXB$_2$	19 ± 7.5	8 ± 2.0	22 ± 8.9	11 ± 5.4	12 ± 2.9†	9.3 ± 3.2†	8.8 ± 3.8	13 ± 4.9	14 ± 4.6	23 ± 6.5*	23 ± 8.4	25 ± 9.2*
AA - LOX Oxylipins												
5-HETE[2,3]	282 ± 39	280 ± 47	211 ± 24	270 ± 53	226 ± 33	246 ± 28†	221 ± 30	219 ± 25	209 ± 16	222 ± 29	224 ± 21	338 ± 62*
8-HETE[2,4]	371 ± 29	353 ± 36	366 ± 40	426 ± 65	371 ± 34	380 ± 39	464 ± 83	363 ± 45	446 ± 71	540 ± 72	406 ± 37	442 ± 75
9-HETE[2]	292 ± 138	256 ± 91	169 ± 41	324 ± 116	139 ± 20	204 ± 62	252 ± 94	147 ± 16	192 ± 33	299 ± 78	163 ± 27	308 ± 142
11-HETE[1]	489 ± 62	380 ± 61	370 ± 35	425 ± 62	408 ± 41	383 ± 55	519 ± 105	360 ± 54*	449 ± 78	501 ± 66	409 ± 48	498 ± 106
12-HETE[2,4]	429 ± 57	384 ± 56	396 ± 57	474 ± 69	346 ± 40	396 ± 60†	482 ± 45	476 ± 61	462 ± 91	528 ± 87	384 ± 33	577 ± 91
15-HETE	351 ± 25	341 ± 44	298 ± 28*	366 ± 46	337 ± 28	364 ± 36	354 ± 41	376 ± 42	344 ± 23	377 ± 43	340 ± 20	397 ± 39
5-oxoETE	122 ± 30	165 ± 55	292 ± 174	142 ± 33	724 ± 455	886± 567*†	580 ± 320	121 ± 29	235 ± 78	181 ± 51	289 ± 184	160 ± 46
12-oxoETE[2]	6.9 ± 2.8	7.5 ± 2.3	8.6 ± 2.4	5.7 ± 1.6	4.4 ± 1.3	6.8 ± 1.6	4.9 ± 2.7	12 ± 3.1	8.1 ± 2.1	11 ± 3.0	11 ± 3.6	11 ± 4.2
15-oxoETE	27 ± 4.9	30 ± 4.1	25 ± 4.8	32 ± 6.4	33 ± 4.9	25 ± 2.0	33 ± 4.7	26 ± 3.1	29 ± 3.6	32 ± 4.6	23 ± 3.4	22 ± 5.0
20oh LTB$_4$	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
LTB$_4$	0 ± 0	0 ± 0	0.6 ± 0.6	0.6 ± 0.5	0 ± 0	1.8± 1.8*†	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0
LTE$_4$[4]	0.8±0.6†	0.5 ± 0.3	1.5 ± 1.1	0.8 ± 0.4	3.1 ± 1.5	1.7 ± 1.7	3.8 ± 1.8	2.0 ± 1.0	2.5 ± 1.6	0.8 ± 0.3	3.6 ± 2.4	3.7 ± 1.9
tetranor 12-HETE	93 ± 13	104 ± 17	71 ± 15†	86 ± 17	74 ± 10	108 ± 23	103 ± 15	93 ± 13	112 ± 19	110 ± 15	78 ± 11	76 ± 22
AA - cP450 Oxylipins												
5,6 DiHETrE	71 ± 6.5	67 ± 7.9	105 ± 26†	85 ± 15	77 ± 14	61 ± 7.6	68 ± 10	54 ± 5.2	62 ± 8.2	70 ± 16	74 ± 22	69 ± 7.6
5,6 EpETrE	0.7 ± 0.5	1.6 ± 0.9	2.5 ± 0.9	3.1 ± 1.2	2.2 ± 1.0	2.3 ± 1.1	1.6 ± 1.1	1.4 ± 1.0	3.3 ± 1.5	3.6 ± 1.8	1.9 ± 0.9	0.5 ± 0.5
8,9 DiHETrE[2]	32 ± 2.0	30 ± 1.9	30 ± 2.3	33 ± 3.0	29 ± 1.8	34 ± 2.4†	33 ± 2.4	31 ± 2.5	30 ± 1.5	32 ± 2.4	29 ± 2.4*	28 ± 3.3*
11,12 DiHETrE[3]	131 ± 10	119 ± 5.9	124 ± 7.8	119 ± 8.1	122± 8.3†	129 ± 10	145 ± 12	118 ± 4.7*	124 ± 8.6	125 ± 6.6	100 ± 5.6*	109 ± 7.1*

11,12 EpETrE	11 ± 1.0	11 ± 1.8	14 ± 3.0	15 ± 2.8	11 ± 1.9	10 ± 1.3†	12 ± 2.7	9.5 ± 1.0	10 ± 1.4	14 ± 2.3	8.3 ± 1.5	16 ± 3.9
14,15 DiHETrE	177 ± 16	163 ± 10	164 ± 12	168 ± 10	167 ± 13	190 ± 13†	186 ± 12	166 ± 12	193 ± 12	172 ± 7.5	159 ± 10	151 ± 6.0*
14,15 EpETrE	41 ± 7.6	32 ± 5.2	33 ± 7.4	37 ± 6.9	33 ± 6.7	46 ± 8.3	36 ± 4.2	31 ± 4.2	39 ± 8.3	35 ± 5.2	39 ± 8.8	54 ± 12
16-HETE[3]	194±27†	137 ± 21	175 ± 39	172 ± 37	114 ± 26*	160 ± 37	124 ± 20	118 ± 19	172 ± 23	212 ± 37*	120 ± 18	133 ± 32
17-HETE	70 ± 8.6	50 ± 4.5*	54 ± 8.8	54 ± 5.1	42 ± 3.6*	54 ± 8.9	56 ± 7.1	50 ± 4.0	63 ± 6.4	64 ± 12	51 ± 5.2	44 ± 5.1
18-HETE[4]	99 ± 7.0	89 ± 6.1†	93 ± 10	97 ± 4.0	76± 3.0*†	87 ± 9.2†	90 ± 3.0	117 ± 9.0*	113± 8.7*	106 ± 8.3	103 ± 5.4	117 ± 13*
20-HETE	517 ± 54	460 ± 52	462 ± 68	495 ± 52	465 ± 43	455 ± 35	475 ± 39	482 ± 51	520 ± 48	497 ± 40	462 ± 33	552 ± 62
AA- Non-Enzymatic Oxylipins												
5-iso PGF2aVI	18 ± 5.3	35 ± 5.7*	20 ± 5.7	23 ± 4.8	29 ± 6.3	23 ± 4.8	26 ± 7.7	35 ± 6.2	32 ± 5.0	30 ± 5.2	16 ± 3.1	20 ± 6.7
ALA - LOX Oxylipins												
9-HOTrE	437±120	299 ± 124	309 ± 128	296 ± 110	415 ± 115	463 ± 166	351 ± 137	451 ± 121	423 ± 141	357 ± 119	302 ± 108	327 ± 179
13-HOTrE	482 ± 62	537 ± 81	541 ± 76	549 ± 78	567 ± 85	732 ± 69*	492 ± 62	413 ± 63	532 ± 106	554 ± 84	424 ± 53	544 ± 88
9 oxoOTrE[3]	41 ± 11	74 ± 15	83 ± 31	154 ± 50*	77 ± 21	110 ± 40	75 ± 22	25 ± 6.8	50 ± 10	118 ± 51	58 ± 21	63 ± 23
ALA - cP450 Oxylipins												
12,13 diHODE	65 ± 16	54 ± 13	50 ± 7.6	68 ± 15	115 ± 50	101 ± 35	79 ± 21	52 ± 10	67 ± 32	85 ± 15	114 ± 51	92 ± 31
12,13 EpODE[2]	23± 3.0†	44± 8.2*†	26 ± 3.3	43 ± 7.2*†	33 ± 5.5	35 ± 6.2	39 ± 8.1	30 ± 5.2	23 ± 3.4	31 ± 8.9	25 ± 4.5*	28 ± 7.3
EPA - COX Oxylipins												
TXB_3	0 ± 0†	0.2 ± 0.2	0.4 ± 0.4	0 ± 0	0 ± 0	0 ± 0	1.7 ± 1.1	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0.9 ± 0.6
EPA - LOX Oxylipins												
5-HEPE[2,3,4]	62 ± 12	85 ± 18	57 ± 10†	96 ± 29†	64 ± 9.4†	61 ± 14†	82 ± 20	113 ± 21	129 ± 17*	146 ± 26*	155 ± 21*	146 ± 33*
8-HEPE[3,4]	2.9 ± 1.5	3.7 ± 2.1	3.4 ± 2.3	4.8 ± 3.2	2.9 ± 1.8†	0.5 ± 0.5	1.0 ± 1.0	6.9 ± 4.6	9.0 ± 4.1	13 ± 4.9*	13 ± 5.3*	0 ± 0
9-HEPE[3,4]	47 ± 7.1	56 ± 10†	61 ± 12†	56 ± 14†	57 ± 7.4†	59 ± 11†	62 ± 18	98 ± 10	117 ± 18*	147 ± 33*	110 ± 16*	129 ± 34*
12-HEPE[2,3,4]	75 ± 15	77 ± 15	59 ± 16†	78 ± 27†	51 ± 7.5†	90 ± 21†	58 ± 7.6	107 ± 17*	137 ± 24*	143 ± 22*	148 ± 16*	204 ± 25*
15-HEPE[3,4]	66 ± 13	48 ± 7.6†	52 ± 11†	67 ± 13†	50 ± 5.0†	83 ± 16†	57 ± 7.7	91 ± 14*	125 ± 18*	118 ± 13*	116 ± 14*	156 ± 21*
EPA - cP450 Oxylipins												
18-HEPE[3,4]	83 ± 16	75 ± 7.3†	68 ± 8.2†	97 ± 16†	69 ± 6.2†	81 ± 10†	104 ± 20	118 ± 14	161 ± 23*	189 ± 33*	203 ± 24*	207 ± 25*
DHA - LOX Oxylipins												

4-HDoHE[2,3,4]	310 ± 70	287 ± 59†	224 ± 39†	347 ± 90†	227 ± 33†	268 ± 49†	218 ± 35	600± 122*	711±128*	793± 127*	899± 115*	1257± 236*
7-HDoHE[3,4]	178 ± 28	144 ± 37†	174 ± 46†	211 ± 58†	165 ± 29†	247 ± 76†	223 ± 44	298 ± 40	418 ± 65*	463 ± 50*	499 ± 76*	471 ± 80*
8-HDoHE[3,4]	111 ± 14	116 ± 20†	108 ± 27†	171 ± 43†	118 ± 16†	169 ± 53†	136 ± 40	301 ± 47*	420 ± 64*	431 ± 60*	458 ± 59*	476 ± 93*
10-HDoHE[2,3,4]	50 ± 8.6	38 ± 7.2†	42 ± 7.4†	56 ± 17†	43 ± 7.2†	52 ± 11†	40 ± 8.2	126 ± 26*	175 ± 37*	157 ± 19*	193 ± 22*	111 ± 19*
11-HDoHE[2,3,4]	85 ± 16	71 ± 12†	84 ± 17†	116 ± 34†	84 ± 14†	107 ± 28†	105 ± 33	169 ± 32	250 ± 47*	259 ± 36*	314 ± 34*	280 ± 51*
13-HDoHE[1,2,3,4]	52 ± 9.4	49 ± 10†	40 ± 8.5†	61 ± 16†	40 ± 7.3†	48 ± 9.0†	39 ± 4.6	143 ± 31*	154 ± 28*	160 ± 30*	167 ± 22*	209 ± 40*
14-HDoHE[2,3,4]	191 ± 58	164 ± 34	123 ± 20†	202 ± 44	124 ± 26†	129 ± 25†	111 ± 18	285 ± 63*	395±115*	342 ± 54*	355 ± 37*	652 ± 123*
16-HDoHE[2,3,4]	99 ± 13	98 ± 14†	94 ± 19†	133 ± 29†	89 ± 11†	101 ± 14†	85 ± 10	238 ± 41*	281 ± 47*	289 ± 35*	336 ± 41*	325 ± 61*
17-HDoHE[3,4]	326 ± 51	301 ± 47†	297 ± 57†	399 ± 96†	289 ± 37†	346 ± 33†	304 ± 30	737± 104*	876±154*	840± 117*	925± 102*	1065± 168*
DHA - cP450 Oxylipins												
19,20 DiHDPE[2,3,4]	946±108	943±114†	896±114†	1093 ± 198†	848 ± 94†	973± 113†	903 ± 94	1248± 107*	1554± 131*	1778± 188*	1832± 153*	1847± 168*
19,20 EpDPE[3,4]	27 ± 6.6	24 ± 5.5	27 ± 11	27 ± 6.7†	25 ± 5.7†	29 ± 5.0†	22 ± 4.1	37 ± 4.5	40 ± 6.6	54 ± 11*	59 ± 14*	66 ± 12*
20-HDoHE[3,4]	203 ± 21	230 ± 29†	214 ± 36†	296 ± 74†	195 ± 31†	193 ± 24†	173 ± 26	482 ± 73*	489 ± 57*	588± 121*	724± 104*	767 ± 168*

[1]Produced by COX and LOX; [2]Sex Effect; [3]Time Effect; [4]Treatment Effect; (--) represents analyte was below limit of quantitation

Table 0-10. Plasma oxylipins for males and females consuming ALA oil treatment grouped by parent PUFA (pM).
(*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
Oxylipin	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
						LA Derived Oxylipins						
Total	26266±2509	30867±4775	26392±2454	36214±7384	35738±8377	26819±2684	29460±4561	52878±15999	25742±5349	28814±4080	33592±6519	25637±4554
LOX	25399±2576	29814±4747	25617±2380	34955±7197	35050±8345	25825±2544	28616±4488	51558±15840	24993±5141	27916±3973	32764±6354	24629±4346
cP450[1]	868±130	1053±186	775±156	1259±253	689±67	994±152	844±137	1320±263	749±219	899±162	828±231	1008±215
						DGLA Derived Oxylipins						
Total	428±108	355±58	377±150	293±29	339±100	286±33	384±88	366±85	415±121	208±25*	470±93	259±23
COX	282±112	173±61	246±142	121±25	194±101*	123±38	227±74	121±63	272±121	50±14*	275±77	69±10
LOX	145±18	181±10	131±21	172±18	145±16	164±16	157±15	246±34*	143±21	158±19	195±34	190±23
						AA Derived Oxylipins						
Total	4130±447	4029±300	3372±295	3857±458	3632±443	3745±311	3376±318	4709±515	3947±855	4012±640	4137±816	4833±1386
COX	776±149	693±119	420±75*	580±71	631±248	559±68	442±76*	745±115	479±48*	632±104	625±119	603±88
LOX	2323±359	2620±210	2044±351	2558±352	2172±346	2197±195	1922±282	3256±428	2594±834	2737±642	2522±670	3458±1329
cP450	1446±148	1241±88	1160±95*	1157±140	1142±96*†	1376±166	1289±51	1261±65	1202±87	1073±46	1270±120	1197±122
cP450 Epoxygenase	487±60	443±30	425±32	422±38	415±58†	527±56	461±51	454±37	471±64	410±24	481±56	465±46
cP450 hydroxylase	960±111	798±80	736±86*	735±116	727±100*	849±122	828±79	807±69	731±83	663±49	788±117	733±78
						Total Omega-6 Derived Oxylipins						
	30927±2695	35359±4860	30231±2625	40462±7831	39780±7965	30940±2612	33312±4488	58065±16387	30204±5507	33132±4418	38330±6869	30854±5689
						ALA Derived Oxylipins						
Total	1257±176	840±232	783±103†	1235±348	942±253	1070±210	1171±337	1050±93	1328±223	1086±207	1617±352	1267±278
LOX	1170±182	751±216	722±98†	1099±328	874±248	984±220	1086±328	911±92	1194±170	926±208	1531±345	1080±229
cP450[1]	88±22	89±26	61±11	136±33	68±8.1	86±14	85±16	139±28	134±74	160±81	86±27	187±67
						EPA Derived Oxylipins						

Total	278±38	392±103	313±60	377±80	283±63	319±73	240±47	559±141	265±35	322±38	320±70	426±94
LOX	210±31	295±75	243±50	297±70	223±63	242±69	170±31	435±127	202±29	246±35	254±66	329±83
DHA Derived Oxylipins												
Total[1]	2005±174	3154±548	2064±267	2867±517	1897±126	2749±615	2013±283	4203±1060	1991±259	2503±400	2510±456	2814±521
LOX[1]	1105±159	1702±414	1067±261	1470±282	980±90	1393±364	1012±230	2373±594	1105±174	1255±262	1425±412	1509±312
cP450[1]	901±60	1452±177	997±53	1397±256	917±66	1356±259	1001±65	1830±499	886±89	1248±173	1085±125	1304±227
cP450 epoxygenase[1]	732±58	1214±161	799±43	1136±220	740±51	1106±219	815±39	1424±372	726±75	1019±155	884±119	1119±199
Total Omega-3 Derived Oxylipins												
	3541±352	4386±692	3160±326	4479±616	3122±377	4138±701	3424±597	5812±1197	3584±491	3911±594	4447±749	4507±729
Total Enzymatically Derived Oxylipins												
COX	1101±216	928±154	699±145*	767±99	854±223	733±67	698±96*	958±160	778±129	734±118	937±155	731±98
LOX	30309±3062	35291±5101	29784±2789	40478±7818	39374±7971	30739±2922	32901±4469	58655±16578	30192±5527	33179±4499	38630±7077	31133±5728
cP450[1]	3371±320	3931±398	3063±249	4029±598	2875±176	3888±362	3289±143	4675±681	3034±425	3456±348	3334±415	3793±567
cP450 Epoxygenase[1]	2174±215	2798±273	2060±203	2952±491	1912±66	2713±352	2205±137	3338±501	2080±358	2488±310	2279±408	2778±462
cP450 hydroxylase	1197±115	1133±137	1003±102	1076±150	964±124	1175±143	1083±102	1337±197	954±99	968±82	1055±113	1015±124
Total Oxylipins	34468±3014	39745±5304	33392±2844	44943±8250	42907±7936	35085±3134	36739±4523	63888±17310	33793±5936	37045±4813	42779±7448	35362±6226

[1]Sex Effect; [2]Time Effect; [3]Treatment Effect

Table 0-11. Plasma oxylipins for males and females consuming DHA oil treatment grouped by parent PUFA and enzymatic production (pM).
(*) Significant difference between baseline and time point for the same treatment. (†) Significant difference between sexes for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 1		Day 3		Day 7		Day 14		Day 28	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
LA Derived Oxylipins												
Total	40247±14125	37542±4494	22278±2591	56738±21471	22280±4270	38500±5461	28144±6388	59075±15324	26679±6548	36374±11377	74303±28276*	62387± 23822
LOX	39601±14042	36337±4360	21623±2542	55690±21554	21468±4230	37249±5378	27344±6327	57783±15348	26221±6572	35289±11381	73681±28301*	61022± 23740
cP450[1]	646±134	1205± 208	655±87	1048±263	812±186	1251±350	801±146	1291±131	458±63	1085±213	622±63	1365±316
DGLA Derived Oxylipins												
Total	328±45	395±73	453± 100*†	303±70	393±114	379±30	450±104	366±44	437±147	352±48	530±119*†	369±63
COX	166±38	190±54	295±97*†	110±29	241±103	151±32	290±105†	142±44	288±142	193±38	358±105*†	167±48
LOX	162±22	206±43	158±20	193±47	152±21	228±19	160±8.8	224±24	149±10	159±25	172±33	203±26
AA Derived Oxylipins												
Total	4169± 1010	4703±722	3070±114	4038±415	3353±237	4930±471	3995±383	4739±514	3618±512	3816±187	3891±577	4696±524
COX	753±227	585±120	374±19*†	634±125	536±72	755±147	659±134	780±100	601±39	714±136	619±180	741±119
LOX	2812±847	3252±683	1814±108	2581±354	1897±195	3272±305	2543±307	3045±460	2255±468	2356±151	2516±511	3134±461
cP450	1132±98	1323±91	1124±34	1231±69	1247±63	1429±193	1238±103	1407±113	1094±113	1190±57	1163±131	1371±92
cP450 Epoxygenase	429±32	534±53	428±30	395±20*	455±21	469±53	430±22	469±47	421±66	401±48*	447±27	411±40*
cP450 Hydroxylase	703±71	789±45	696±35	836±74	792±51	960±145	808±115	937±68	673±64	789±50	716±139	959±73
Total Omega-6 Derived Oxylipins												
	44852±14300	42810±4915	25885±2600	61210±21410	26114±4396	43924±5893	32692±6395	64305±15283	30796±6241	40651±11314	78846±28674*	67553± 23862
ALA Derived Oxylipins												
Total	1085±292	992±266	992±142	950±312	1198±296	974±198	1364±176	963±215	968±309	889±230	1075±394	1043±372
LOX	1005±281	835±220	932±141	847±295	1152±295	830±196	1272±189	827±198	882±270	705±151	1019±391	869±353
cP450[1]	80±18	157±46	60±8.6	102±23	46±5.7	144±49	93±29	136±26	86±53	184±90	56±4.7	173±54
EPA Derived Oxylipins												

Total	411±83	322±55	442±78†	623±92*	543±106†	854±89*	714±168*	789±95*	661±92*	815±112*	689±117*†	1029±67*
LOX	279±73	242±46	325±69	504±79*	414±81†	655±60*	552±115*	579±76*	474±69*	598±96*	509±108*†	773±74*
DHA Derived Oxylipins												
Total[1]	2144±368	2577±399	3811± 632*	5517±936*	4761±838*	7003±997*	5036±788*	7071±983*	5736±1020*	7595±635*	6262± 1107*	8776±847*
LOX[1]	1206±282	1318±236	2292±522	3503±742*	2846±608*†	4681±1000*	3067±442*	4274±690*	3408±613*	4745±491*	3882±705*†	5799±790*
cP450[1]	938±113	1259±182	1518±161*	2014±227*	1915±258*	2322±132*	1969±360*	2797±422*	2328±462*	2851±215*	2380±420*	2977±177*
cP450 Epoxygenase[1]	780±112	1070±144	1104±132	1465±149*	1468±182*	1783±141*	1579±240*	2043±286*	1672±298*	2069±146*	1609±210*	2216±168*
Total Omega-3 Derived Oxylipins												
	3640±687	3891±527	5246±774	7090± 1097*	6502±1022*	8831±1212*	7114±900*	8823±998*	7364±1216*	9299±753*	8026± 1504*	10848± 945*
Total Enzymatically Produced Oxylipins												
COX	951±248	824±147	783±134	917±137	906±63	1091±191	1054±100	1128±118	1018±136	1106±129	1146±203	1167±171*
LOX	45011±14311	42155±4939	27072±2971	63258±22175	27868±4964	46804±6599	34882±6539	66635±15769	33306±6116	43801±11115	81728±28387*	71698±23737*
cP450[1]	2926±325	4022±436	3475±239	4514±462	4150±433*	5344±564*	4263±485*	5840±468*	4152±489*	5527±472*	4400±560*	6142±521*
cP450 Epoxygenase[1]	1935±265	2965±371	2247±205	3009±399	2782±307*	3647±419*	2902±249*	3939±291*	2636±268*	3740±416*	2734±222*	4165±528*
cP450 Hydroxylase	991±77	1056±92	1228±89	1505±201*	1368±137*	1697±230*	1361±287	1902±229*	1515±233*	1787±105*	1665±385*	1977±146*
Total Oxylipins	48491± 14488	46701± 5382	31132± 3170	68301± 22320	32619±5060	52757±6912	39809±6827	73129±15871	38163±6054	49950±11091	86872±28129*	78401±23957*

[1]Sex Effect; [2]Time Effect; [3]Treatment Effect

Table 0-12. Average dietary consumption of participants at baseline and day 21 of each treatment phase.
Data represents sexes combined. (†) Significant difference between treatments for the same time point ($p < 0.05$). Values represent the mean±SEM.

Dietary Component	Day 0		Day 21	
	DHA	ALA	DHA	ALA
Kcal (g)	2216 ± 176	2498 ± 243	2137 ± 126	2291 ± 252
Carbohydrate (g)	272 ± 18	309 ± 30	251 ± 17	287 ± 30
Fibre (g)	24 ± 3.7	28 ± 4.5	23 ± 2.7	25 ± 4.1
Protein (g)	95 ± 7.4	104 ± 7.9	98 ± 7.6	93 ± 6.9
Fat (g)	82 ± 10	85 ± 14	80 ± 6.1	84 ± 15
Total Sugars (g)	93 ± 12	110 ± 21	85 ± 16	98 ± 18
Added Sugars (g)	40 ± 9.1	53 ± 16	35 ± 9.9	51 ± 16
Saturated Fat (g)	26 ± 3.9	30 ± 6.4	25 ± 2.9	28 ± 7.0
Trans Fat (g)	0.98 ± 0.23	0.79 ± 0.20	1.0 ± 0.32	1.0 ± 0.20
Monounsaturated Fat (g)	33 ± 4.0	36 ± 5.2	29 ± 2.7	32 ± 5.4
PUFA (g)	17 ± 2.1	16 ± 1.9	17 ± 1.9	17 ± 2.2
Cholesterol (mg)	405 ± 49	354 ± 40	337 ± 42	263 ± 39
Vitamin A (mcg)	899 ± 141	1016 ± 166	767 ± 93	845 ± 198
Vitamin D (IU)	175 ± 29	148 ± 18	165 ± 42	126 ± 28
Vitamin E (mg)	9.6 ± 1.5	11 ± 1.9	7.6 ± 0.5	10 ± 1.5
Thiamin (mg)	1.5 ± 0.13	2.1 ± 0.29	1.8 ± 0.24	1.9 ± 0.27
Riboflavin (mg)	2.3 ± 0.17†	2.6 ± 0.20	2.2 ± 0.12	2.1 ± 0.22
Niacin (NE)	41 ± 3.3	47 ± 3.2	44 ± 3.6	42 ± 3.6
B6 (mg)	1.9 ± 0.19	2.0 ± 0.20	2.2 ± 0.11	2.0 ± 0.15
Folate (mcg)	521 ± 65	518 ± 58	454 ± 41	486 ± 65
B12 (mcg)	4.6 ± 0.46	4.6 ± 0.58	3.8 ± 0.53	3.8 ± 0.73
Vitamin C (mg)	126 ± 23	180 ± 36	168 ± 31	131 ± 17
Sodium (mg)	3449 ± 655	4079 ± 996	3290 ± 686	3512 ± 854
Potassium (mg)	3158 ± 271	3400 ± 291	3206 ± 185	3220 ± 285
Calcium (mg)	1032 ± 127	1011 ± 70	957 ± 151	900 ± 120

Iron (mg)	15 ± 1.4	17 ± 1.4	15 ± 1.4	17 ± 2.0
Food Energy (KJ)	9217 ± 738	10413 ± 1024	8867 ± 538	9519 ± 1060
Water (g)	2147 ± 367	2352 ± 264	2015 ± 344	2044 ± 316
Omega-6 (g)	13 ± 1.6	12 ± 1.6	14 ± 1.7	12 ± 1.6
LA (g)	13 ± 1.6	12 ± 1.6	14 ± 1.7	13 ± 1.6
Omega-3 (g)	1.6 ± 0.24	1.6 ± 0.30	1.63 ± 0.21	1.84 ± 0.35
ALA (g)	1.4 ± 0.21	1.5 ± 0.30	1.5 ± 0.20	1.8 ± 0.35
EPA+DHA (g)	0.15 ± 0.07	0.05 ± 0.01	0.07 ± 0.02	0.06 ± 0.01
Omega-6/Omega-3	9.3 ± 1.1	8.3 ± 0.58	9.2 ± 1.2	7.8 ± 1.0
Vitamin K (mcg)	146 ± 42	154 ± 37	143 ± 29	215 ± 83
Beta-Carotene (mcg)	4870 ± 1173	5770 ± 1738	3777 ± 691	4935 ± 2197
Lycopene (mcg)	2719 ± 1774	4428 ± 1198	3334 ± 1698	3896 ± 2284
Magnesium (mg)	517 ± 176	371 ± 34	349 ± 24	369 ± 38
Manganese (mg)	4.0 ± 0.69	4.5 ± 0.67	3.8 ± 0.32	4.2 ± 0.60
Phosphorous (mg)	1375 ± 116	1427 ± 125	1459 ± 109	1337 ± 111
Selenium (mcg)	107 ± 9	111 ± 14	115 ± 13	91 ± 9.6
Zinc (mg)	11 ± 0.74	13 ± 1.6	12 ± 0.85	12 ± 0.93

Table 0-13. Participant reported activity levels based on average weekly activity.
Data represents sexes combined. (*) Significant difference between from baseline for the same time point ($p < 0.05$). (†) Significant difference between treatments for the same time point ($p < 0.05$). Values represent the mean±SEM.

	Day 0		Day 21		
	Fish	**Flax**	**Fish**	**Flax**	**Sex Effect**
Vigorous Activity (days/week)	2 ± 0.5	3 ± 0.6	3 ± 0.5	3 ± 0.6	
Vigorous Activity (min/day)	65 ± 13	42 ± 6.9	47 ± 7.5	44 ± 9.7	
Moderate Activity (days/week)	4 ± 0.6	3 ± 0.7	4 ± 0.7	4 ± 0.6	
Moderate Activity (min/day)	66 ± 12	60 ± 14	52 ± 9.9	57 ± 16	0.0187
Walking (days/week)	5 ± 0.6	5 ± 0.5	4 ± 0.6†	6 ± 0.5	
Walking (min/day)	81 ± 26	92 ± 27	141 ± 43*	85 ± 27	
Sitting (min/day)	338 ± 41	330 ± 37	276 ± 41	313 ± 43	

Table 0-14 Calculated time where plateau for select omega-6 and omega-3 fatty acids was reached for males and females.
Plateaus are calculated for females, males, and both sexes combined for fish and flax treatments. Values represent day from baseline when plateau concentration was observed.

	Fish			Flax		
Fatty Acid	**Female**	**Male**	**Sex Combined**	**Female**	**Male**	**Sex Combined**
C18:2ω6	--	--	--	--	--	--
C18:3ω6	--	5	5	--	--	--
C20:3ω6	--	--	--	--	--	--
C20:4ω6	--	--	--	--	--	--
C22:5ω6	4	--	--	--	--	--
C18:3ω3	--	--	--	5	6	--
C20:5ω3	5	8	--	--	--	--
C22:5ω3	--	--	--	5	--	--
C22:6ω3	6	8	--	--	--	--

(--) Indicates no plateau observed.

Table 0-15. Calculated time where plateau for ALA derived oxylipins in flax oil treatment was reached for males and females. *Plateaus are calculated for females, males, and both sexes combined for flax treatments. Values represent day from baseline when plateau was observed.*

	Flax		
Analyte	**Female**	**Male**	**Sex Combined**
12,13-diHODE	--	--	--
12,13-EpODE	5	--	--
9-HOTrE	--	--	--
13-HOTrE	--	--	--
9-oxoOTrE	5	--	--
Total ALA Oxylipins	--	--	--
Total Omega-3 Oxylipins	--	--	--

(--) Indicates no plateau observed.

Table 0-16. Calculated time where plateau for EPA and DHA derived oxylipins in fish oil treatment was reached for males and females.
Plateaus are calculated for females, males, and both sexes combined for fish treatments. Values represent day from baseline when plateau was observed.

	Fish		
Analyte	**Female**	**Male**	**Sex Combined**
TXB_3	--	--	--
5-HEPE	--	--	--
8-HEPE	--	--	--
9-HEPE	5	--	--
12-HEPE	6	--	--
15-HEPE	5	6	--
18-HEPE	6	--	--
Total EPA Oxylipins	6	--	--
4-HDoHE	6	--	--
7-HDoHE	--	--	--
10-HDoHE	--	6	--
11-HDoHE	6	--	--
13-HDoHE	6	--	--
14-HDoHE	5	--	--
17-HDoHE	6	--	--
19,20-DiHDoPE	6	7	--
19,20-EpDPE	--	6	--
20-HDoHE	7	--	--
8-HDoHE	6	6	--
16-HDoHE	7	--	--
Total DHA Oxylipins	7	--	-
Total Omega-3 Oxylipins	7	--	-

(--) Indicates no plateau observed.