Effect of dietary soy protein and oils containing omega-3 fatty acids on disease and renal oxylipins in polycystic kidney disease

by

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ABSTRACT

Polycystic kidney disease (PKD) is the most common genetic renal disease for which no curative treatment is currently available. Oxylipins are bioactive lipids formed from polyunsaturated fatty acids (PUFA), having roles in proliferation and inflammation, processes involved in PKD progression. Oxylipin alterations have been reported in non-orthologous models of PKD. In these models, dietary soy protein and n-3 PUFA have shown benefits. However, recent studies in an orthologous model and humans have yielded conflicting results.

To examine whether benefit of dietary treatments in non-orthologous models are replicated in orthologous models, and to investigate the role of oxylipins, four independent studies were conducted using PCK rats, $Pkd2^{WS25/-}$(Pkd2) mice and $Mx1Cre^{+}Pkd1^{flox/flox}$(Pkd1) mice. Animals were given either casein or soy protein and one of soy, flax or fish oil in the diet. Generally, fish oil yielded no beneficial effects on disease but resulted in enlarged kidneys in all three models and higher kidney water content in PCK rats and Pkd2 mice. Flax oil resulted in lower water content in Pkd1 mice kidneys, but higher water content, size and cyst in PCK rat kidneys. Dietary soy protein improved proteinuria and urine pH, only in PCK rats. Targeted lipidomics was used to screen for over 150 oxylipins of which 50-55 were detected in each model. Cyclooxygenase derived oxylipins were consistently higher in diseased compared to normal animals and cytochrome P450 epoxygenase derived oxylipins were lower only in PCK rats. Fish oil was more effective than flax oil in reducing n-6 oxylipins. Fish oil also resulted in the highest levels of EPA and DHA oxylipins, whereas flax oil had the highest ALA oxylipins. Soy protein compared to casein resulted in higher LA oxylipins. AA oxylipins were higher in females compared to males, while oxylipins from other PUFA were either higher in males or unaffected.

Therefore, in contrast to studies in non-orthologous models, these studies do not support dietary advice to increase soy protein or oils enriched in n-3 PUFA in early PKD. Disease associated oxylipin alterations, unique effects of soy protein on LA and sex on AA oxylipins are novel findings that demand further research.

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DEDICATION

I dedicate this thesis to

My parents, ***Devassy Gopuran*** and ***Celina Devassy***

and

my wife, ***Jasmi Gopuran***

THESIS ORGANIZATION

This thesis was prepared following a manuscript format, and it is composed of four manuscripts. The thesis begins with a general introduction (chapter 1) followed by literature review (chapter 2). The literature review includes manuscript 1 (chapter 2.2) which is a review paper on oxylipin synthesis and functions, co-authored by me and published in Advances in Nutrition. Chapter 2 also includes review of other relevant literature on diet effects on renal oxylipins and polycystic kidney disease. Hypothesis and objectives are presented in chapter 3. Manuscripts 2, 3 and 4 appear as chapters 4, 6 and 7 respectively. Chapter 5 contains results from studies that are not included in manuscript 2. Manuscript 2 is published in PLoS One journal, and manuscript 3 and 4 are being prepared for submission. The thesis is concluded with a general discussion, conclusions and future directions (chapters 8 and 9).

CONTRIBUTIONS OF AUTHORS

Manuscript 1:

Advances in our understanding of oxylipins derived from dietary polyunsaturated fatty acids.

Gabbs M, Leng S, **Devassy JG**, Monirujjaman M, Aukema HM.

JG Devassy prepared the 7 figures used in this paper. Additionally, he contributed extensively to the text and tables in the paper, checked references for accuracy and responded to reviewer's concerns during final submission. M Gabbs and S Leng contributed the material from their master's proposal and contributed to the text and the tables. M Monirujjaman wrote the part about the oxylipins from AdA. Aukema HM conceived the idea of the paper, wrote and corrected the manuscript and approved its final submission.

Manuscript 2:

Lack of Benefit of Early Intervention with Dietary Flax and Fish Oil and Soy Protein in Orthologous Rodent Models of Human Hereditary Polycystic Kidney Disease

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JG Devassy with everyday help from T Yamaguchi conducted animal feeding, tissue collection, and urine sample analysis of PCK rats. JG Devassy also conducted the histology and statistical analysis in the PCK rat study. JG Devassy captured the histology pictures of Pkd2 mice. JG Devassy prepared the figures used in the paper. Additionally, breeding and colony expansion of Pkd2 mice was done by T Yamaguchi T and JG Devassy. M Monirujjaman conducted the animal feeding phase and sample analysis of Pkd2 mice. M Gabbs helped with the animal

feeding and sample collection. HM Aukema developed the concept and designed experiments, wrote the final manuscript and responded to reviewer comments.

Manuscript 3:

Distinct bioactive lipids alterations in diverse models of cystic kidney diseases

Monirujjaman M, **Devassy JG***, Yamaguchi T, Gabbs M, Zhou J, Ravandi A, Aukema HM.

****Equally contributing first author***

This manuscript (chapter 6) is a major portion of the manuscript under preparation for submission to JASN. Of the four studies included in the manuscript, JG Devassy and T Yamaguchi conducted the breeding and colony expansion and feeding phase of three studies (PCK rats and two Pkd1 mice studies). JG Devassy conducted all the procedures for lipid extraction and oxylipin analysis and statistical procedures from these three studies and prepared result tables for the manuscript. M Monirujjaman conducted the animal feeding, lipid extraction, oxylipin analysis and statistical analysis of the Pkd2 mice study and prepared the manuscript. M Gabbs helped with the animal feeding and sample preparation for oxylipin analysis. J Zhou provided Pkd1 mice and A Ravandi provided the mass-spectrometric facilities for the oxylipin analysis. HM Aukema developed the concept and designed experiments, corrected the final manuscript and approved its submission.

Manuscript 4:

Distinct effects of dietary flax compared to fish oil, soy protein compared to casein, and sex on the renal oxylipin profile in models of polycystic kidney disease

Devassy JG, Yamaguchi T*, Monirujjaman M, Gabbs M, Zhou J, Ravandi A, Aukema HM

****Equally contributing first author***

JG Devassy and T Yamaguchi conducted the animal feeding for 3 of 4 studies. JG Devassy conducted all the procedures and statistical tests and wrote the manuscript. M Monirujjaman conducted the Pkd2 mice study. Gabbs M helped with the animal feeding and sample preparation for oxylipin analysis. J Zhou provided Pkd1 mice and A Ravandi provided the mass-spectrometric facilities for the oxylipin analysis. HM Aukema developed the concept and designed experiments, corrected the final manuscript and approved its submission.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
AdA	Adrenic acid
ADPKD	Autosomal dominant polycystic kidney disease
AIN	American Institute of Nutrition
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
ARPKD	Autosomal recessive polycystic kidney disease
ASA	Acetylsalicylic acid
AT	Aspirin- Triggered
cAMP	Cyclic adenosine monophosphate
COX	Cyclooxygenase
$cPLA_2$	Cytosolic phospholipase A_2
CYP	Cytochrome P450
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DiHDoHE	Dihydroxy-docosahexaenoic acid
DiHDPE	Dihydroxy-docosapentaenoic acid
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
EpDPE	Epoxy-docosapentaenoic acid
EpEDE	Epoxy-eicosadienoic acid
EpETE	Epoxy-eicosatetraenoic acid
EpETrE	Epoxy-eicosatrienoic acid
EpODE	Epoxy-octadecadienoic acid
EpOME	Epoxy-octadecenoic acid
FLAP	5-lipoxygenase activating protein
EPA	Eicosapentaenoic acid
ESRD	End-stage renal disease
Ex	Eoxin
GLA	Gamma-linolenic acid
GLM	General linear model
GFR	Glomerular filtration rate
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
HpETE	Hydroperoxy-eicosatetraenoic acid
HpETrE	Hydroperoxy-eicosatrienoic acid
HpEPE	Hydroperoxy-eicosapentaenoic acid
HpODE	Hydroperoxy-octadecadienoic acid
HpOTrE	Hydroperoxy-octadecatrienoic acid
Hx	Hepoxilin
LA	Linoleic acid
LOX	Lipoxygenase
Lt	Leukotriene
Lx	Lipoxin
MaR	Maresin
oxo-DoHE	Oxo-Docosahexaenoic acid
oxo-EPE	Oxo-Eicosapentaenoic acid
oxo-ETE	Oxo-Eicosatetraenoic acid
oxo-ODE	Oxo-Octadecadienoic acid
oxo-OTrE	Oxo-Octadecatrienoic acid
PBS	Phosphate buffered Saline
PD	Protectin
PG	Prostaglandin
PGEM	Prostaglandin E metabolite
pI:pC	Polyinosinic polycytidylic acid
PKD	Polycystic kidney disease

PMN	Polymorphonuclear leukocyte
Rv	Resolvin
SE	Standard error
sEH	Soluble epoxide hydrolase
TriHOME	Trihydroxy-octadecenoic acid
Trx	Trioxilin
Tx	Thromboxane

Chapter 1

1.1 General Introduction

Polycystic kidney disease (PKD) is the most common inherited renal disease and the fourth leading cause of end stage renal disease (1). This disease is characterized by progressive enlargement of the kidney due to expansion of cysts, resulting in renal insufficiency and several other complications including hypertension (1). The underlying genetic causes of the disease have been discovered (2, 3). Despite a great deal of research that went into this disease in the last 4 decades, a curative treatment option has not yet emerged. Dietary interventions with soy protein and oils rich in n-3 PUFA have shown promise in reducing the disease in models with cystic kidney disease (4-10), based on which recommendations for increasing these dietary elements were issued to PKD patients (11-13). However, these experiments were conducted on non-orthologous models of PKD where the genetic cause of the disease is different from those in humans (14, 15). A recent study in an orthologous model, and a short study in humans have shown a lack of benefit from fish oil and soy protein (16, 17) suggesting that these interventions may be not beneficial in human conditions. Therefore, it is important to confirm the beneficial effects in true models of PKD and in humans before recommendations can be provided to patients.

Recent observations from models of cystic kidney disease showed that renal oxylipins are altered in this disease (10, 18). Oxylipins are oxygenated metabolites of polyunsaturated fatty acids (PUFA) generated via cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) pathways (19). These bioactive lipids participate in a variety of signalling activities including inflammation and proliferation (20, 21). Although disease associated alterations in a

limited number of oxylipins have been reported (10, 18), a detailed report of oxylipin changes in PKD is lacking.

It is known that dietary flax oil and fish oil affects oxylipin synthesis, and benefits disease in PKD (8, 10). However, there have been no studies investigating flax and fish oil side by side in the same model for its effects on oxylipins in the kidney, or in any other tissue. Soy protein also modulates enzymes involved in oxylipin production in PKD (10), but it is not clear if there are disease independent effects on oxylipins. There have been reports of differences in disease between male and female in PKD (22-24), but whether such differences are reflected in, or mediated through oxylipins is not known. Therefore, the research presented herein aims to evaluate the effect of dietary flax oil, fish oil and soy protein and sex effects on disease progression and the renal oxylipin profile in three orthologous models of PKD.

Chapter 2

Literature Review

2.1 Polycystic kidney disease – the most common genetic kidney disease

2.1.1 Overview

There are several types of cystic kidney diseases identified in humans, characterised by cysts initiating from nephrons and collecting tubules, but varying in origin and pathogenesis. Cyst initiation is thought to be caused by abnormal signalling in the primary cilia found on the surface of the tubular epithelium of nephrons (25). Primary cilia functions as a mechano-, osmotic- and chemical sensor, which transmit further signals to control proliferation and differentiation, thus maintaining the normal tubular structure. Malfunctioning of these pathways leads to unregulated proliferation and subsequent cyst formation (2).

Polycystic kidney disease (PKD) is one such inherited renal disease where functions of cilia are compromised due to genetic mutations in one or more genes encoding the proteins in cilia (1). There are two major forms of PKD, autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). Both forms are characterized by expansion of renal cysts yielding progressive enlargement of the kidney and renal insufficiency along with several other complications (1).

Another related genetic disease in which renal cysts are present is nephronophthisis (NPHP). NPHP is a group of autosomal recessive kidney disorders, which presents with renal cysts and fibrosis and often is associated with polyuria and polydipsia (26, 27). About 20 mutations in genes encoding the NPHP protein family have been linked to this disease. Occurrence of this disease varies largely (~1:50,000 in Canada (27)). Because phenotypic expression of the disease in models of NPHP is similar to PKD, a large proportion of the previous studies in polycystic kidney disease were carried out on models of NPHP. However, the underlying genetic causes of disease are different from human PKD. In the current thesis,

orthologous models of polycystic kidney disease were used and the results are compared with previous results from NPHP or related models.

2.1.2 ADPKD

ADPKD is referred to as adult PKD due to the slow progression of the disease. Growth of renal cysts results in progressive enlargement of kidneys, with renal failure occurring after the fifth decade of life (28). Occurrence of the disease is approximately 1:800 to 1:1,000 people and accounts for 7-10% of all cases of end-stage renal disease (28-30). Despite large growth of the kidneys, glomerular filtration rate (GFR) is conserved in these patients until ages 30-40, and is followed by a quick and linear decline (29, 31). Half of the ADPKD patients will require regular dialysis or a renal transplant by the age of 60 (32). Hypertension is the most common clinical manifestation of ADPKD and is often accompanied by acute abdominal or flank pain (33-35). Hypertension is present in more than half of the patients even before impairment of renal function, and in almost all patients by the time they reach end-stage renal disease (ESRD) (33, 36). Extrarenal manifestations include cyst growth in liver, pancreas, thyroid, subarachnoid, and seminal vesicles (28, 37). Intracranial aneurysms and nephrolithiasis are additional complications often seen in 20-40% of these patients (38, 39).

Genetically, ADPKD is inherited in a dominant fashion with nearly complete penetrance. Mutations in the *PKD1* and *PKD2* genes, encoding the proteins polycystin-1 and -2 respectively, cause the cyst formation (2). About 85% of ADPKD is caused by mutations in the *PKD1* gene and the remaining 15% are caused by *PKD2* mutation (32). Although mutations in both genes produce phenotypically similar presentations, patients with *PKD1* mutation generally have higher number of renal cysts and rapid progression to ESRD (40). There is variability in the

progression of disease among patients. This has been attributed to several factors such as a 'second hit' (normal allele of the gene sustaining a mutation later in life) (34), maternal vs paternal inheritance (41), role for modifier genes (42) and involvement of unknown genes (43).

2.1.3 ARPKD

ARPKD is the form of PKD that typically affects younger patients (44) where many patients die due to respiratory failure in the perinatal stage (45). Disease is characterised by dilation of collecting ducts due to cysts and hepatic fibrosis (46). Estimated prevalence is 1 in 20,000 live births (47). Age of onset and clinical presentations of ARPKD are highly variable, with most cases detected in utero and some not until after childhood (44, 48, 49). Most in-utero cases display symptoms of lack of amniotic fluid such as pulmonary hypoplasia, extremity abnormalities, unusual facial appearances, and deformities of the spine and die during the neonatal period (44). In cases of delayed presentation, symptoms are usually due to congenital hepatic fibrosis (49).

Genetically, ARPKD is caused by mutation at a single locus, the Polycystic Kidney and Hepatic Disease 1 gene (*PKHD1*) (3). *PKHD1* gene codes for the protein fibrocystin, which plays a crucial role in formation of cilia structure (50). There are several hundred mutations identified in this gene, some more lethal than others (51), explaining the variability in clinical presentation (52).

2.1.4 Pathophysiology

The key abnormality causing cyst formation in both ADPAKD and ARPKD is associated with cilia mediated signalling (2). Primary cilium is a hair-like structure present on the

surface of most cells of the body (2, 53). In kidney, primary cilia are found projecting from the apical surface of the renal epithelium into the tubular lumen, generating a flow-induced increase in intracellular calcium in response to fluid flow over renal epithelia (53, 54). Polycystin-1, polycystin-2, and fibrocystin (coded by *PKD1*, *PKD 2* and *PKHD1* respectively*)* are proteins present within the cilium structure and basal body of renal tubular epithelia (55). Defects in these proteins caused by mutations in their respective genes causes malformation of cilia (56), which in turn results in the loss of its mechano-sensory function. This leads to disruption of calcium signaling, contributing to cyst formation (55). Although the precise mechanisms of how impaired cilia lead to cyst development are unknown, disruption of one of several pathways regulated by primary cilium are thought to be involved. These signalling pathways include intracellular calcium, Hedgehog pathway, Wnt/β-catenin, cyclic adenosine monophosphate (cAMP), or planar cell polarity (2, 57, 58). Disturbances in these cascades cause dedifferentiation of cystic epithelia, increased proliferation, increased apoptosis, and loss of resorptive capacity (59, 60). Eventually, cyst growth and expansion leads to compression of renal vessels and intrarenal ischemia. This activates the renin-angiotensin-aldosterone system, and in turn accelerates cyst expansion and renal fibrosis (61).

2.1.5 Current treatment strategies

Despite the great deal of research that went into the field, there exists no curative treatment option for the patients suffering from PKD. Existing clinical care focuses on managing the symptoms, mainly bleeding and pain. A major part of the treatment is controlling blood pressure. Recent discoveries of molecules that may slow disease progression has attained great

attention. Such strategies include use of angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, vasopressin receptor 2 antagonists and mTOR inhibitors (62). These treatments have shown some benefits in reducing blood pressure and improving renal function, but not consistently (62).

In the absence of effective treatment options, lifestyle modifications including dietary changes are of utmost importance in slowing down the disease. There are several dietary recommendations by various PKD organisations (11-13). Lowering protein (63), and sodium intake (64), increasing intake of water (65), soy protein (10) and oils containing n-3 polyunsaturated fatty acids (10) have been shown to be beneficial in slowing disease progression. The effects of dietary flax oil, fish oil and soy protein on disease will be discussed in detail in the section 2.4.

2.1.6 Transition to next section

Previous studies in models of cystic kidney disease have revealed involvement of polyunsaturated fatty acids (PUFA). Tissue levels of PUFA and enzymes metabolizing PUFA were altered in diseased kidneys compared to normal. Some recent studies have shown that levels of a class of bioactive lipid molecules, oxylipins, are also altered in renal tissues of cystic kidney disease models. Oxylipins are signaling lipids, which plays crucial role in physiological processes in health and disease. In the next section (2.2) is a manuscript that reviews the literature on oxylipins and their functions in health and disease.

2.2 Advances in our understanding of oxylipins derived from dietary polyunsaturated fatty acids

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[4]Abbreviations used [follows Lipid Maps format (66, 67): 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-eicosapentaenoic acid; oxo-ODE, oxo-octadecadienoic acid; oxo-OTrE, oxo-octadecatrienoic acid; PD, protectin [also called neuroprotectin (NPD) in the brain]; PG, prostaglandin; PGEM, prostaglandin E metabolite; PMN, polymorphonuclear leukocyte; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; Rv, resolvin; SDA, stearidonic acid; sEH, soluble epoxide hydrolase; TriHOME, trihydroxy-octadecenoic acid; Trx, trioxilin; Tx, thromboxane

2.2.1 Abstract

Oxylipins formed from polyunsaturated fatty acids (PUFA) 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.

2.2.2 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.

2.2.3 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. (68-70). 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 ($cPLA_2$) (71). 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 (72, 73). 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 (74). 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* (75, 76).

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 (77-88).

2.2.3.1 Cyclooxygenase

The first oxylipin generation pathway involves cyclooxygenase (COX) enzymes, which convert PUFA into prostanoids – i.e. prostaglandins and thromboxanes (77-79). 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 (89, 90). 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) (77, 79). 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 (79). 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] (91-94).

2.2.3.2 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 (78, 82). An alternative nomenclature is to use the gene names to describe the LOX enzymes (82).

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 (86). 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 (81, 83). Hepoxilins also are formed from 12-HpETE (88)and eoxins from 15-HpETE (95). 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.

2.2.3.3 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 (78). 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 (78, 80, 84, 85). In addition, they

may also enter cells and mediate effects intracellularly by modulating transcription factors and ion channels (80).

2.2.4 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 (96, 97), the presence of oxylipins from other PUFA demonstrates that they can be released in sufficient quantities for oxylipin production. Pathways are shown in Figures 2.1-2.7 and described by PUFA precursor below.

2.2.4.1 N-6 PUFA

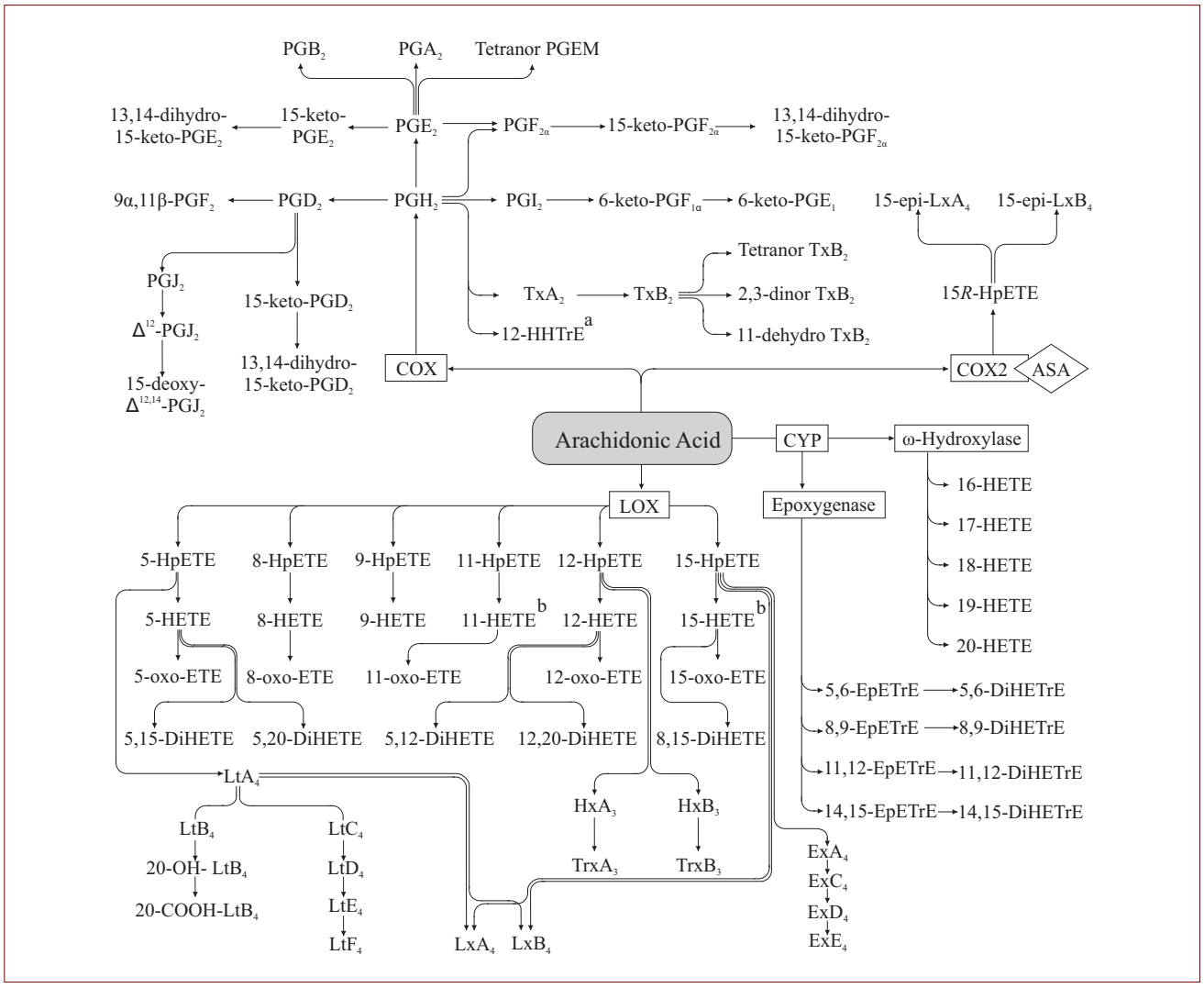
Arachidonic Acid – *Figure 2.1*. AA produces 2-series oxylipins via the COX pathway, initially resulting in formation of PGG_2 and PGH_2, which is then rapidly converted to other prostaglandins (e.g. $PGF_{2\alpha}$) and thromboxanes (e.g. TxA_2) via specific prostaglandin and thromboxane synthases (87). 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 (78, 98, 99).

AA also produces oxylipins via the LOX pathway, resulting in HpETE, (e.g. 12-HpETE), which are further rapidly converted to hydroxy fatty acids via glutathione peroxidase (100). 5-, 12-, 15-HETE are the most commonly described HETE in mammals, although 8-, 9- and 11-

HETE also are produced, and sometimes in greater amounts (101, 102). The 11- or 15-HETE isomers also can be produced via COX activity, as indicated above (91, 92). The HETE can be further converted to oxo-ETE via dehydrogenase activity (103, 104), or to DiHETE, via further COX (e.g. 5,11-DiHETE), LOX (e.g. 5,15-DiHETE) or CYP ω-hydroxylase (e.g. 5,20-DiHETE) activity (105, 106). In addition, the HpETE formed via LOX can be metabolized via several other routes: 5-HpETE can be further converted to 4-series leukotrienes (e.g. LtC_4), via 5-LOX after activation by FLAP; 12-HpETE can be isomerized to hepoxilins (e.g. HxB_3) and subsequently converted to trioxilins [e.g. trioxilin B_3 ($TrxB_3$)] (88, 107); and 15-HpETE can be converted to eoxins (e.g. ExC_4) (95). As well, lipoxins (e.g. LxA_4) can be formed from 5- or 15-HpETE via further LOX activity (108-110). Epi-Lx (e.g. 15-epi-LxA_4) formation can also be initiated by aspirin acetylated or nitrosylated COX2 and 5-LOX (111-113). AA also can be converted non-enzymatically to HETE (114) and isoprostanes (e.g. iso-$PGF_{2\alpha}$) (115). The latter are often used as a marker of oxidative stress in vivo; for further discussion of these non-enzymatic oxylipins, see review in (115).

AA metabolism via CYP ω-hydroxylase activity results in the formation of HETE with the hydroxy group being at the omega or methyl end of the fatty acid (e.g. 20-HETE), while CYP epoxygenase activity yields epoxy fatty acids (e.g. 14,15-EpETrE), which can be converted to dihydroxy fatty acids (e.g. 14,15-DiHETE), via sEH activity, as reviewed in (80, 84, 85). Formation of other HETE (e.g. 13-HETE) may be mediated via CYP bisallylic hydroxylase activity (116, 117), but the importance of this pathway is less known.

Figure 2.1. AA derived oxylipins.

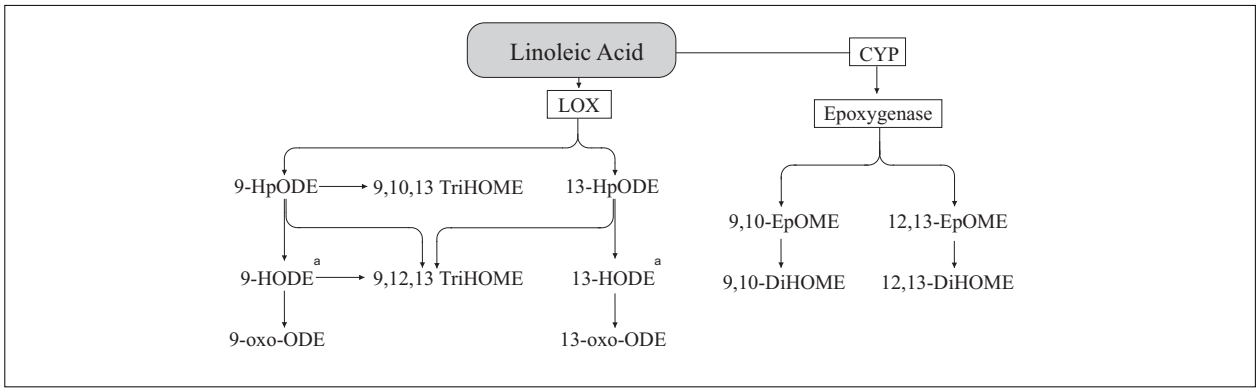


[a]There is also evidence for thromboxane synthase-independent production of HHTrE (118)
[b]Also produced via the COX pathway (91, 92)

Linoleic Acid –Figure 2.2. Even though the size of the literature for LA oxylipins is markedly smaller than for most other oxylipins (especially AA oxylipins), they are usually present in tissues and blood in higher amounts than oxylipins derived from any other PUFA (119-121). LA produces oxylipins through the LOX pathway, resulting in hydroperoxy fatty acids, which are rapidly converted to hydroxy fatty acids [e.g. 13-hydroxy-octadecadienoic acid (HODE)],

which can be further metabolized to keto fatty acids [e.g. 13-oxo-octadecadienoic acid (13-oxo-ODE) (122, 123). LA also can be metabolized via the epoxygenase activity of CYP, resulting in epoxygenated fatty acids [e.g. 9,10-epoxy-octadecenic acid (9,10-EpOME)], which are metabolized via sEH activity to form dihydroxy fatty acids [e.g. 9,10-dihydroxy-octadecenoic acid (9,10-DiHOME)] (124). Further, LA can be converted to trihydroxy fatty acids [e.g. 9,10,13-trihydroxy-octadecenoic acid (9,10,13-TriHOME)] potentially by sequential metabolism of LOX and epoxygenase activity and/or auto-oxidation (125). Several other LA oxylipins also can be produced non-enzymatically (e.g. 9-HODE) (126). There also are reports that the formation of a small amount of the LA oxylipins may be mediated via COX (e.g. 9-HODE) (94, 127) or CYP bisallylic hydroxylation (e.g. 17-HODE) (116, 117, 128) activity; the relative importance of these pathways remain to be elucidated.

Figure 2.2. LA derived oxylipins.



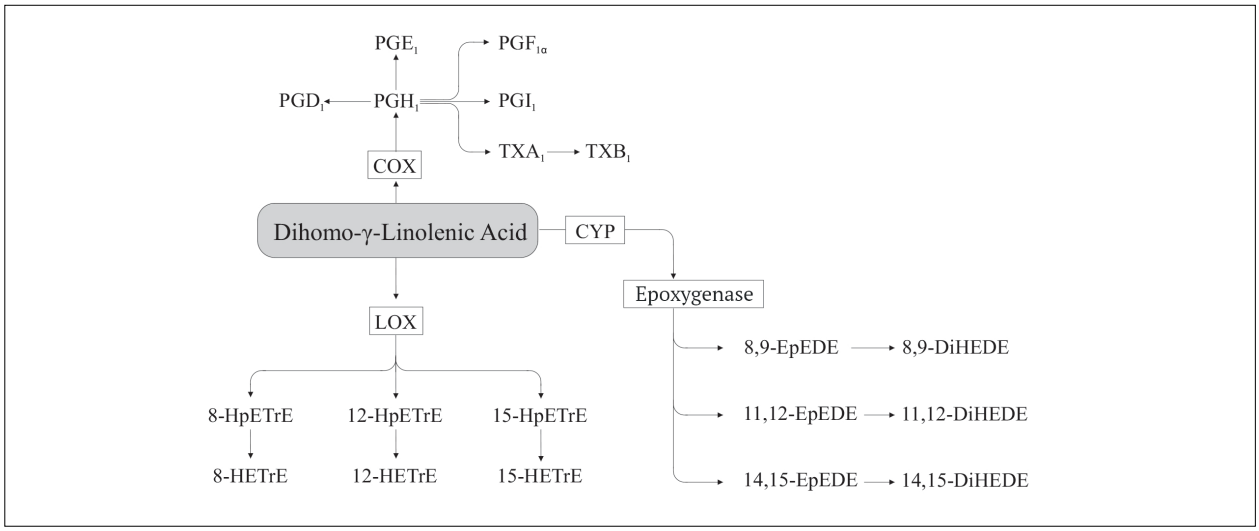
[a]Also produced via the COX pathway (94, 127)

γ-Linolenic Acid. GLA can be converted via LOX to 10- and 13-hydroxy-octadecatrienoic acid(γ) [13-HOTrE(γ)] (129)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 (130). Other

oxylipins derived from GLA (e.g. 6-HOTrEγ) have been reported to be synthesized in vitro in a patent application (131). Note that oxylipins derived from GLA are distinguished from ALA oxylipins with the use of the γ notation.

Dihomo-γ-Linolenic Acid – *Figure 2.3.* DGLA can be converted via COX to 1-series prostaglandins (e.g. PGI_1) and thromboxanes (e.g. TxA_1) (89, 132, 133), via LOX to yield hydroperoxy (e.g. 15-HpETrE) and hydroxy fatty acids [e.g. 15-hydroxy-eicosatrienoic acid (15-HETrE)] (134-139), 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) (135, 136, 140).

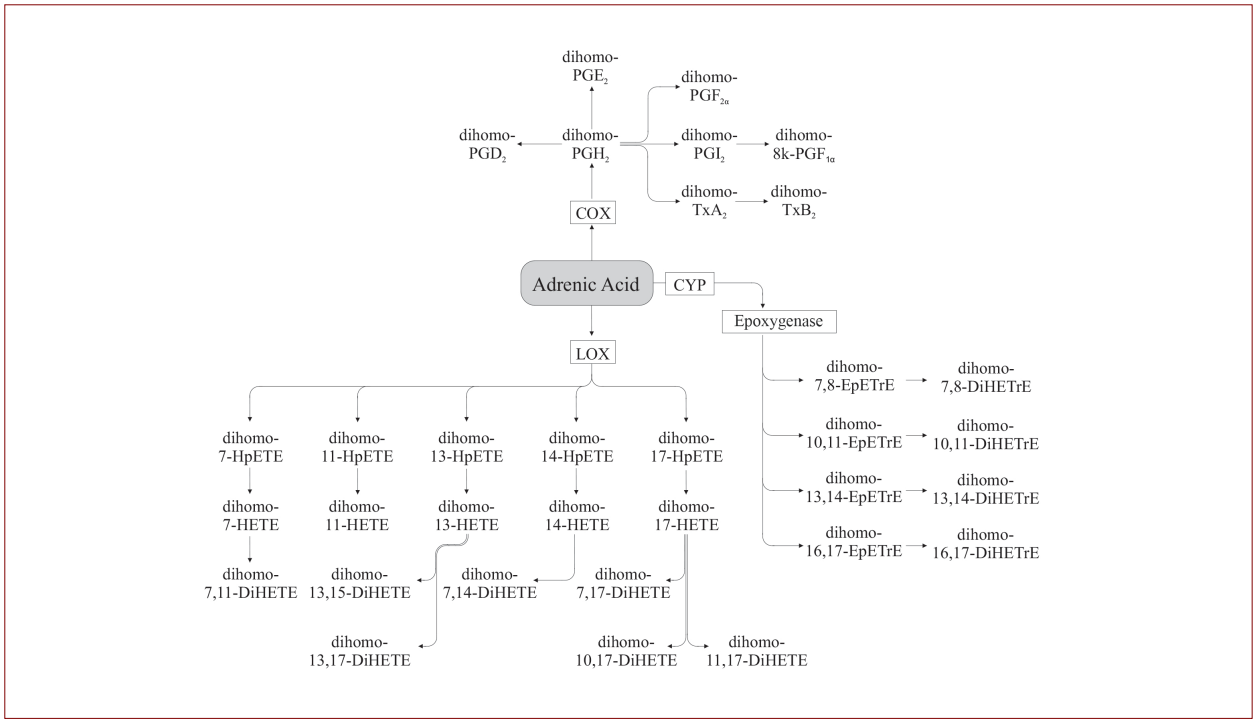
Figure 2.3. DGLA derived oxylipins.



Adrenic Acid – *Figure 2.4.* AdA can be metabolized by COX into dihomo-prostaglandins such as dihomo-PGE_2, dihomo-TxB_2, and dihomo-PGI_2 (141-146). 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) (143-145), 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) (143).

Figure 2.4. AdA derived oxylipins.



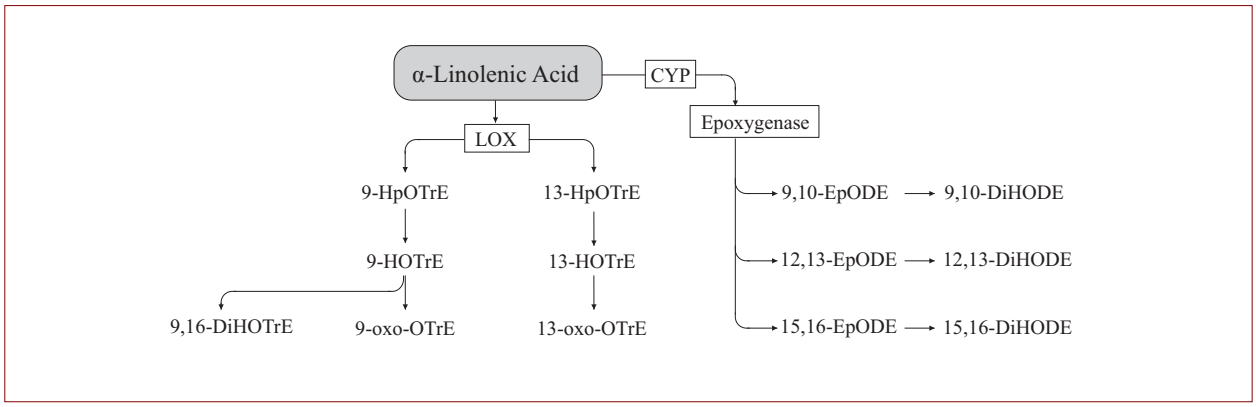
[a]Also can be formed from dihomo-7-HETE (143).

2.2.4.2 N-3 PUFA

α-Linolenic Acid – *Figure 2.5*. 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)] (147). 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 (94). ALA also can be metabolized via CYP epoxygenase activity,

resulting in epoxygenated fatty acids, (e.g. 12,13-EpODE) (130), which can be further converted to dihydroxy fatty acids [e.g. 12,13-dihydroxy-octadecadienoic acid (12,13-DiHODE)] via sEH activity (120). Other ALA metabolites that have been reported include 18-HOTrE from ALA via CYP activity (85), 9,16-DiHOTrE via LOX activity (147) and 12-HOTrE via COX2 activity (94).

Figure 2.5. ALA derived oxylipins.

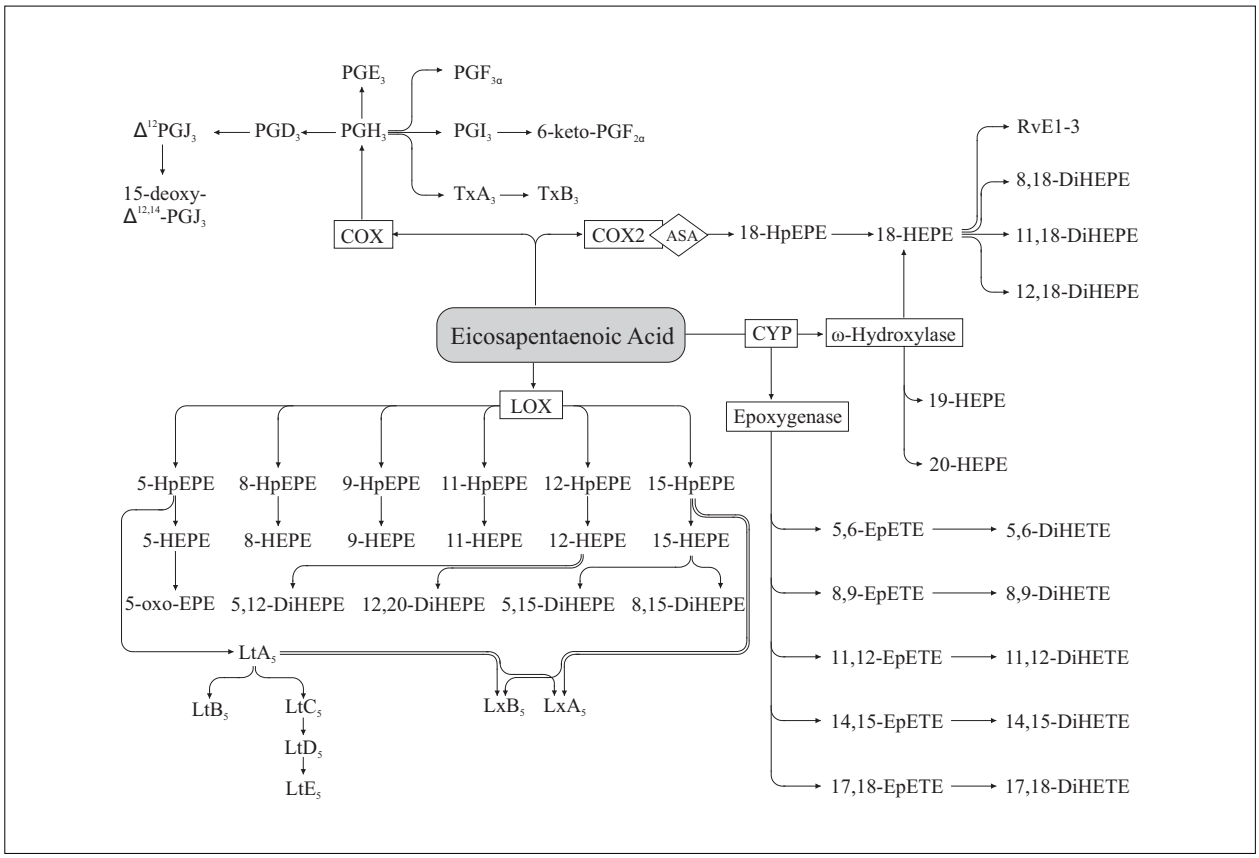


Stearidonic Acid. Oxylipins derived from SDA (e.g. 13- hydroxy-octadecatetraenoic acid) have been reported to be produced in vitro in a patent application (131).

Eicosapentaenoic Acid – *Figure 2. 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) (90). EPA compared to AA is generally a poorer substrate for COX, particularly for the COX1 isoform (148). 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 (90, 149, 150), and 5-series leukotrienes (e.g. LtB_5) via combined 5-LOX and FLAP activity (150, 151). HEPE such as 5-HEPE also can be metabolized to dihydroxy-eicosapentaenoic acids (DiHEPE) such as 5,12-DiHEPE(152)or to keto fatty acids such as 5-oxo-EPE(153). Metabolites of other HEPE

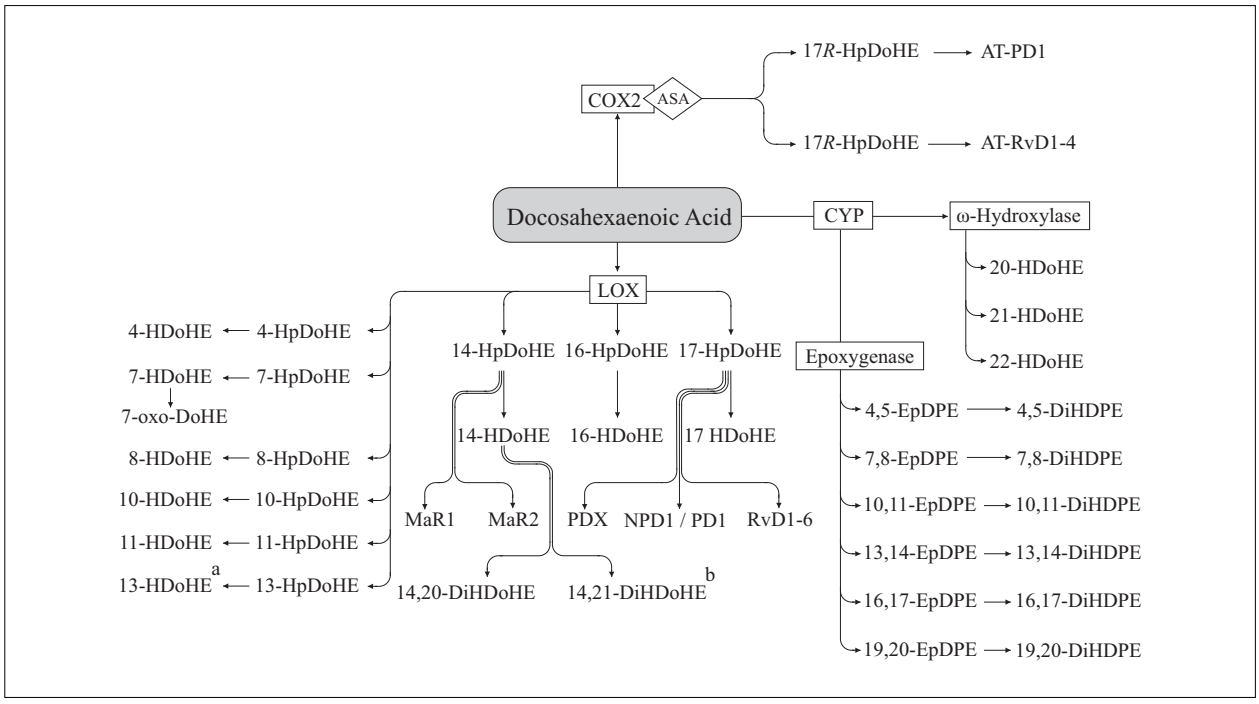
isomers are likely to be present, but few have been identified. Hydroxy fatty acids from EPA with hydroxy groups on the 18-20-carbon positions also are formed via ω-hydroxylase activity of the CYP pathway (e.g. 18-HEPE) (154, 155). The 18-HEPE formed via this pathway (as well as by acetylated COX2) can be further converted to the E-series resolvins [e.g. resolvin E1 (RvE1)] via 5-LOX activity (106, 109, 156). EPA can also produce epoxy fatty acids (e.g. 14,15-EpETE) via CYP epoxygenase activity (157), which can be further converted to dihydroxy fatty acids (e.g. 14,15-DiHETE) by sEH (158). As with AA and LA, bisallylic hydroxylation of EPA can also yield HEPE such as 10-HEPE (159).

Figure 2.6. EPA derived oxylipins.



Docosahexaenoic Acid – *Figure 2.7.* DHA can be metabolized via the LOX pathway to hydroxy fatty acids (e.g. 4-HDoHE), with a hydroperoxy intermediate (e.g. 4-HpDoHE) (160). The hydroperoxy 14-HpDoHE can be further metabolized to form maresins (e.g. MaR1) (161), 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 (162) while PDX is formed via double LOX activity (83). 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) (93, 163, 164). DHA also has been shown to yield hydroxy fatty acids non-enzymatically (e.g. 8-HDoHE)(165, 166)and 13-HDoHE can be formed via COX2 (93). Recent studies provide evidence that HDoHE also can be metabolized to dihydroxy (e.g. 14,20-DiHDoHE) (167) and keto fatty acids (e.g. 7-oxo-DoHE) (168) with more likely to be demonstrated in the future. Oxylipins can be produced from DHA via CYP epoxygenase activity, yielding epoxy fatty acids (e.g. 16,17-EpDPE) (157, 160), which can be converted to dihydroxy fatty acids (16,17-DiHDPE) via sEH (158). CYP ω-hydroxylase activity produces HDoHE with hydroxy groups near the methyl end of DHA (e.g. 21-HDoHE) (160).

Figure 2.7. DHA derived oxylipins.



[a]Also produced via the COX pathway (93)
[b]Also may be formed from 21-HDoHE (169, 170)

2.2.5 Oxylipin Functions

Oxylipins have a wide range of functions, many of which are still being elucidated. In addition, oxylipins derived from different pathways, as well as different substrate PUFA can have similar or opposing effects, necessitating knowledge of the overall oxylipin profile in order to understand their overall biological effects. Their functions are many, including apoptosis, tissue repair, blood clotting, cell proliferation, blood vessel permeability, pain, inflammation, immune actions and blood pressure regulation (78, 171). General functions of oxylipins are described below and examples of functions are provided in Tables 2.1-2.7.

Table 2.1. 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 (172)
	Inhibits Ca^{2+}-stimulated ATPase activity of Walker-256 tumor microsomal membranes (173)
	Represses insulin-like growth factor-I gene expression in C6 rat glioma cells (174)
PGB_2	Mediates mesenteric vascular dose-dependent vasodilatory and vasoconstrictory effects in animal models (175)
	Elevates blood pressure, tracheal segment pressure and bronchial resistance in guinea pigs (176)
PGD_2	Inhibits induced apoptosis in human articular chondrocytes (177)
	Inhibits murine lung inflammation (178)
	Promotes sleeping behavior (179)
	Regulates body temperature in rodent models (180, 181)
	Inhibits tumor cell proliferation in human cells and rodent model (182)
	Pro-inflammatory at nanomolar concentrations and anti-inflammatory at micromolar concentrations [reviewed in (98)]
	Inhibits human neutrophil activation in vitro (183, 184)
	Causes apoptosis of human eosinophils (185)
	Activates human eosinophils (186)
	Inhibits human platelet aggregation (187, 188)
PGE_2	Vasodilates cat cerebral arterioles (189)
	Potentiates human platelet aggregation at lower concentrations and inhibits aggregation at a higher concentrations (190)
	Induces human colon cancer cell growth (191)

	Stimulates IL-10 production in bone marrow-derived dendritic cells in murine model (192)
	Mediates lung inflammation in human cells (193)
15-keto-PGE_2	Activates PPARγ to enhance adipogenesis of murine 3T3-L1 cells (194)
6-keto-$PGF_{1\alpha}$	Stable degradation product of PGI_2 and useful marker of PGI_2 in humans (195, 196)
9α,11β-PGF_2	Activates murine eosinophils (197)
$PGF_{2\alpha}$	Mediates inflammatory tachycardia in the mouse (198)
	Initiates parturition in the mouse (199)
	Vasoconstricts rat brain arterioles (200)
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 (201)
PGI_2	Inhibits ADP-induced hamster platelet aggregation (202)
	Induces coronary vasodilation in dogs (203)
	Inhibits adhesion of human eosinophils to lung endothelial monolayers and transendothelial migration (204)
	Inhibits erythrocyte adhesion to bovine aortic endothelial cells (205)
PGJ_2	Causes apoptosis of human eosinophils (185)
	Induces respiratory burst in human eosinophils (186)
Δ^{12}-PGJ_2	Releases eosinophils from guinea pig bone marrow and induces respiratory burst in human eosinophils (186)
	Causes apoptosis of human eosinophils and neutrophils (185)
15-deoxy-$\Delta^{12,14}$-PGJ_2	Inhibits induced apoptosis in human articular chondrocytes (177)
	Anti-inflammatory via inhibition of NF-κB activation in human and monkey cell culture (206)
	Causes apoptosis of human eosinophils and neutrophils (185)
	Induces respiratory burst in human eosinophils (186)
	Reduces the apoptosis in activated human and murine T-lymphocytes (207)
TxA_2	Mediates inflammatory tachycardia in the mouse (198)
	Causes irreversible platelet aggregation in human platelet rich plasma (208)
	Stimulates mitogenesis of coronary artery smooth muscle cells in guinea pig model (209)
	Mediates hypertension in hypertensive rats (210)
	Vasoconstricts rabbit aorta (211)
TxB_2	Has a weak bronchoactive effect in guinea pigs and dogs (212)
	Increases systemic vascular resistance but does not cause platelet aggregation in dogs (213)
	Chemotactic in human peripheral PMN (214)
2,3-dinor-TxB_2	Marker of thromboxane synthesis in urine of rats (215, 216)
	Possible urinary marker of acute myocardial infarction in humans (217)
11-dehydro-TxB_2	Plasma and urinary marker of thromboxane synthesis in human and rabbit models (99, 218, 219)
	Possible urinary marker of acute myocardial infarction in humans (217)
B. LOX oxylipins	
5,15-DiHETE	Possesses weak human neutrophil and eosinophil chemotactic activity (220)
8,15-DiHETE	Possesses weak human eosinophil chemotactic activity (220)
	Exhibits chemotactic activity comparable to that of LtB_4 for human PMN (221)
12,20-DiHETE	Activates cholesterol ester hydrolysis in human vasculature (222)

Eoxins	Eoxin C_4, D_4 and E_4 all increase permeability of endothelial cell monolayer from human eosinophils and mast cells in vitro (95)
5-HETE	Inhibits the clonal proliferation of chick embryo fibroblasts and granulocytic progenitors (223)
	Stimulates human eosinophil chemotaxis and chemokinesis (224)
	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (225, 226)
	Induces human neutrophil degranulation (227)
	Inhibits PGI_2 production in porcine coronary artery endothelial cells (228)
	Inhibit selenium-induced apoptosis in human prostate cancer cells; 12- and 15-HETE have no effect (229)
	Stimulates proliferation of human cancer cells at low concentrations (230)
	Promotes bovine neutrophil chemotaxis in vitro more potently than 5-HEPE (231)
5-HpETE	Inhibits human platelet aggregation similarly to 5-HpEPE, but less potently than 12- or 15-HpETE (232)
5-oxo-ETE	Stimulates human neutrophils and eosinophils (153, 233)
	Inhibits selenium-induced apoptosis in human prostate cancer cells, with half the potency of 5-HETE (229)
	Stimulates proliferation of human cancer cells in low concentrations and inhibits proliferation at higher concentrations (230)
	Promotes chemotaxis and raises cytosolic calcium levels in human neutrophils; more potent than 5-HETE, 15-oxo-ETE and 5,15-DiHETE (234)
	Stimulates human neutrophils more potently than 5-HETE (234)
	Does not inhibit LOX enzyme activity (cf. 12- and 15-oxo-ETE) in vitro (235)
8-HETE	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (225)
	Promotes wound healing via epithelial cell migration in rat cornea (102)
	Induces differentiation of murine 3T3-L1 pre-adipocytes (236)
9-HETE	Stimulates human eosinophil chemotaxis and chemokinesis (224)
	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (225)

11-HETE	Stimulates human eosinophil chemotaxis and chemokinesis (224) Stimulates human neutrophil chemokinesis and enhances chemotactic responses (225, 226) Inhibits human vascular smooth muscle cell proliferation (237)
11-oxo-ETE	Inhibits human colorectal adenocarcinoma epithelial and umbilical vein endothelial cell proliferation in culture (238)
12-HETE	Stimulates human neutrophil chemokinesis and enhances chemotactic responses (225) Induces human neutrophil degranulation (225) Increases rat heart mitochondrial calcium and nitric oxide, leading to oxidative stress and apoptosis (239) Increases monocyte adhesion to human endothelial cells leading to aortic fatty streak formation (240, 241) Enhances tumor cell adhesion to endothelial cells in mice (242) Enhances thrombin-induced aggregation (243), but suppresses collagen-induced aggregation of bovine platelets (244) Inhibits U-46619-induced aggregation of human platelets (245, 246) Reduces ADP-induced aggregation of mouse platelets (247) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (205)
12-HpETE	Inhibits human platelet aggregation similarly to 12-HpEPE, and more potently than 5- or 15-HpETE (232, 248)
12-oxo-ETE	Selectively inhibits LOX enzyme activity in vitro (235) Activates human neutrophils (249)
15-HETE	Exhibits vasodilation or vasoconstriction in isolated arteries from guinea pig, rabbit, rat and human depending on species and conditions (250) Activates $PPAR_{\gamma}$ in human and $PPAR_{\beta/\delta}$ in mouse (251, 252) Inhibits human PMN migration across cytokine-activated endothelium in vitro (253) Inhibits degranulation and superoxide production in stimulated human PMN (254) Mediates hypoxia-induced rabbit pulmonary hypertension (255) Enhances thrombin-induced human platelet aggregation (256) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (205)
15-HpETE	Exhibits vasodilation or vasoconstriction in isolated arteries from guinea pig, rabbit, rat and human depending on species and conditions (250) Stimulates erythrocyte adhesion to bovine aortic endothelial cells (205) Induces migration of monocyte-like HL-60 cells across a human endothelial cell monolayer (257) Induces loss of rat cardiomyocyte membrane integrity (258) Inhibits human platelet aggregation similarly to 15-HpEPE, but less potently than 12-HpETE (232)
15-oxo-ETE	Selectively inhibits human LOX enzyme activity in vitro (235) Inhibits human vascular vein endothelial cell proliferation (259) Prevents apoptosis of rat pulmonary arterial smooth muscle cells (260)
HxA_3	Activates human neutrophils (261) Recruits human PMN to the site of inflammation (262) Promotes murine 3T3-L1 preadipocyte differentiation (263)
HxB_3	Promotes murine 3T3-L1 preadipocyte differentiation (263)
LtB_4	Releases human PMN lysosomal enzymes (264)

	Induces human PMN chemotaxis and aggregation (265, 266)
	Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (267)
	Promotes chemotaxis of bovine neutrophils, more potently than LtB_5 (231)
20-OH-LtB_4	Stimulates human neutrophil migration, but less potently than LtB_4 (268)
	Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (267)
20-COOH-LtB_4	Stimulates human neutrophil migration, but less potently than LtB_4 (268)
	Stimulates guinea pig lung strip contraction, but less potently than LtC_4 (267)
LtC_4	Causes guinea pig uterine and lung contractions (269)
	Stimulates guinea pig lung strip contraction, more potently than LtB_4 (267)
	Mediates human skin inflammation (270)
	Increases permeability of endothelial cell monolayers from human eosinophils and mast cells in vitro (95)
	Contracts guinea pig lung parenchymal strips and ileal tissues, with similar potency to LtC_5 (271)
LtD_4	Enhances responsiveness to histamine in bovine airway smooth muscle (272)
	Causes guinea pig uterine and lung contraction (269)
	Mediates human skin inflammation (270)
	Increases permeability of endothelial cell monolayer monolayers from human eosinophils and mast cells in vitro (95)
LtE_4	Causes guinea pig uterine and lung contraction (269)
	Coronary constrictor in the in situ pig heart (273)
LtF_4	Induces bronchoconstriction in guinea pig, but less active than LtD_4 (274)
LxA_4	Inhibits LtB_4-induced human PMN activation (275)
	Stimulates human monocyte migration and adhesion (276)
	Inhibits zymosan A-induced peritonitis in mice (277)
	Promotes corneal epithelial cell wound healing in mice (278)
	Increases renal plasma flow and glomerular filtration rate in the rat (279)
	Stimulates phospholipid remodeling without causing aggregation in human neutrophils (280)
	Antagonizes LtD_4-induced lowering of glomerular filtration rate in rat (281)
	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) (282)
	Inhibits proliferation of human A549 cells, but less potently than 15-epi LxA_4, 15-epi LxB_4 or LxB_4 (111)
LxB_4	Stimulates human monocyte migration and adhesion (276)
	Decreases renal plasma flow and glomerular filtration rate in the rat (279)
	Inhibits zymosan A-induced peritonitis in mice (277)
	Stimulates phospholipid remodeling without causing aggregation in human neutrophils (280)
	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) (282)
	Inhibits proliferation of human A549 cells, but less potently than 15-epi LxB_5 (111)
	Inhibits leukocyte-endothelium interactions in mice (283)
	Blocks reactive oxygen species generation in human endothelial cells (284)

	Stimulates human monocyte chemotaxis (285)
	Inhibits proliferation of human A549 cells, but less potently than 15-epi LxB_5 (111)
15-epi LxA_4	Inhibits proliferation of human A549 cells, more potently than 15-epi LxA_4 or LxB_4 (111)
15-epi LxB_4	Inhibits proliferation of human A549 cells, more potently than 15-epi LxA_4 or LxB_4 (111)
	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer(286)
	Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels(287)
C. CYP oxylipins	
5,6-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (286)
	Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers(288)
	Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (287)

8,9-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (286) Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers (288) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (287) Relaxes porcine coronary artery with similar potency as its EpETrE isomer (289)
11,12-DiHETrE	Vasodilates pre-constricted pressurized mouse arteries more potently than its EpETrE isomer (286) Vasodilates isolated canine coronary arterioles more potently than EpETrE isomers (288) Hyperpolarizes rat vascular smooth muscle from rat small coronary arteries by activating BK channels (287) Most potent PPARα activator in a monkey COS-7 cell expression system when compared to other DiHETrE and EpETrE isomers (290) Stimulates metastasis and escape from tumor dormancy in several murine tumor models (291)
14,15-DiHETrE	Vasodilatory effects in intestinal microcirculation in rat model (292) Promotes angiogenesis by stimulating endothelial cell proliferation in vitro and angiogenesis in vivo in murine model (293) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (288) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (286)
5,6-EpETrE	Promotes angiogenesis by stimulating endothelial cell proliferation in vitro and angiogenesis in vivo (293) Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine models (294) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation (295) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (288) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (286)
8,9-EpETrE	Vasodilatory effects in intestinal microcirculation (292) Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine models (294) Inhibits vascular inflammation distinct from its vasodilatory effects by inhibiting nuclear factor-κB and IκB kinase in murine model (296) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation(295) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (288) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (286) Relaxes porcine coronary artery with similar potency as its DiHETrE isomer (289) Enhances angiogenesis and tumor progression in murine model (297)

11,12-EpETrE	Dilates coronary microvessels with similar potency to other EpETrE isomers as well as EpETE and EpDPE isomers in canine and porcine model (294) Attenuates cell apoptosis in rat heart myocytes after hypoxia and re-oxygenation (295) Vasodilates U46619-preconstricted bovine coronary artery rings, more potently than 14,15-DiHETrE (298) Vasodilates isolated canine coronary arterioles less potently than DiHETrE isomers (288) Vasodilates pre-constricted pressurized mouse arteries less potently than its DiHETrE isomer (286) Antinociceptive effect in thermally produced tail-flick response in rats, while other regioisomers were not effective at same dose (299) Enhances angiogenesis and tumor progression (297)
14,15-EpETrE	Induces vasodilation in isolated rabbit kidney (300) Inhibits human leukocyte activation (301) Decreases intracranial pressure in a rabbit model of stroke (301)
16-HETE	Inhibits rabbit proximal tubule ATPase activity, but has no renal vasodilatory activity (300)
17-HETE	Induces vasodilation in isolated rabbit kidney (300)
18-HETE	Reduces pressure in rabbit-perfused kidneys (302) Induces vasodilation in canine renal arteries (303) Stimulates rat renal Na^+/K^+-ATPase (304)
19-HETE	Reduces pressure in rabbit-perfused kidneys (302) Induces vasoconstriction in canine renal arteries and porcine coronary arteries (303, 305)
20-HETE	Reduces pressure in rabbit-perfused kidneys (302) Stimulates inflammatory cytokine production in human endothelial cells (306) Stimulates proliferation of rat vascular smooth muscle cells (307)

Table 2.2. Examples of LA derived oxylipin functions

A. LOX oxylipins	
9-HODE	Induces endoplasmic reticulum stress in human macrophages (308)
	Inhibits proliferation and induces apoptosis in human U937 cells (309)
	Pro-inflammatory in skin under oxidative conditions in human (310)
	Induces maturation, scavenger receptor expression and activates PPARγ-dependent transcription in human monocytes (311)
	Does not inhibit tumor cell adhesion to endothelial cells (cf. 13-HODE) in mice (242)
9-oxo-ODE	Activates PPARγ-dependent transcription in human monocytes (as does 9-HODE and -HpODE) (311)
13-HODE	Prevents platelets from adhering to human vascular endothelium (312)
	Decreases thrombin-induced platelet adherence to other platelets and to the endothelial cells *in vitro* (313)
	Induces maturation, scavenger receptor expression and activates PPARγ-dependent transcription in human monocytes (311)
	Inhibits proliferation of hyperproliferative skin in guinea pigs (314)
	Inhibits tumor cell adhesion to endothelial cells (242)
	Inhibits the secretion and assembly of triacylglycerol-rich lipoprotein particles in vitro (315)
	Inhibits human neutrophil production of LtB_4 in vitro (137)
13-HpODE	Relaxes canine circumflex and splenic arteries, similarly to 13-HODE (316)
	Relaxes human pulmonary arteries (250)
13-oxo-ODE	Reduces inflammation in human colonic epithelial cells (317)
	Does not inhibit tumor cell adhesion to endothelial cells (cf. 13-HODE) in mice (242)
	Does not inhibit LOX enzyme activity (cf. 12- and 15-oxo-ETE) in vitro (235)
	Activates PPARγ-dependent transcription in human monocytes (as does 13-HODE and -HpODE) (311)
B. CYP oxylipins	
9,10-DiHOME	Decreases left ventricular developed pressure recovery and increases coronary resistance following ischemia/reperfusion in mouse heart (318)
	Causes mitochondrial dysfunction, leading to cell death in rabbit renal proximal tubular cells, while parent epoxy compound is not toxic (319)
12,13-DiHOME	Causes mitochondrial dysfunction, leading to cell death in rabbit renal proximal tubular cells, while parent epoxy compound is not toxic (319)
	Causes acute respiratory distress syndrome in mice; more toxic than its epoxy parent (320)
	Lacks protective effect of 12,13-EpOME in rabbit renal proximal tubular cells exposed to hypoxia/reoxygenation (321)
9,10-EpOME	Inhibits mitochondrial respiration in perfused rat lung (322)
	Relaxes rat stomach smooth muscle and uncouples mitochondrial respiration (323)
	Induces canine heart failure when injected intravenously (324)
	Inhibits growth of normal and transformed human cells in culture (325)
	Induces vasoconstriction in isolated perfused cat carotid arteries (326)

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 (321) Induces vasoconstriction in isolated perfused cat carotid arteries (326) Induces dysfunction in isolated rabbit renal cortical mitochondria, while 12,13-DiHOME does not (327)

Table 2.3. Examples of DGLA derived oxylipin functions

A. COX oxylipins	
PGD_1	Activates pro-inflammatory receptor CRTH2/DP2 in human kidney cells (cf. PGE_1) (328)
	Inhibits human platelet aggregation, but is 100-fold less potent than PGD_2 or PGD_3 (187)
PGE_1	Does not activate pro-inflammatory receptor CRTH2/DP2 in human kidney cells (cf. PGD_1) (328)
	Reduces healing time of lower limb ulcers in human patients (329)
	Alleviates neurological deteriorations of diabetic rats (330)
	Vasodilates rat coronary and systemic circulation (331)
	Stimulates peripheral blood flow in humans with peripheral arterial disease (332)
	Reduces pulmonary hypertension in patients with pulmonary arterial hypertension (333)
	Inhibits human platelet aggregation (188, 334)
13,14-dihydro-PGE_1	Inhibits human platelet aggregation with similar potency to PGE_1 (334)
B. LOX oxylipins	
12-HETrE	Enhances delayed-type hypersensitivity in guinea pig model (335)
	Inhibits human platelet aggregation (336)
15-HETrE	Inhibits epidermal hyperproliferation in guinea pig skin (134, 337)
	Inhibits formation of pro-inflammatory LtB_4 in human neutrophils (137)
	Inhibits cellular growth and AA metabolism in human prostatic adenocarcinoma cells (338)

Table 2.4. 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 (144) No contractile activity in vascular and non-vascular smooth muscle tissue at levels that PGE_2 had significant activity (144)
Dihomo-PGI_2	Inhibits thrombin-induced human platelet aggregation, but is 100-fold less potent than PGI_2 (142) Stimulates cAMP production in rabbit renal medullary interstitial cells, but 100 times less potently than PGI_2 (144)
Dihomo-TxA_2	No contractile activity in rabbit aorta (144, 211)[compared to constrictory effect of TxA_2]
B. CYP oxylipins	
Dihomo-7,8-, Dihomo-10,11-, Dihomo-13,14-, Dihomo-16,17-EpETrE	Induce vasorelaxation in bovine coronary arterial rings (146) Dilate canine and porcine coronary microvessels with similar potency to other dihomo-EpETE isomers as well as EpETrE and EpEPE isomers (294)
Dihomo-16,17-EpETrE	Causes concentration-related relaxations in pre-constricted bovine adrenal cortical arteries (143)

Table 2.5. Examples of ALA derived oxylipin functions

A. COX oxylipins	
9-HOTrE	Associated with glomerular hypertrophy in obese rats (339)
9,16-diHOTrE	Inhibits prostaglandin synthesis from COX1 and collagen induced human platelet aggregation (147)
13-HOTrE	Suppresses IL-1β induced expression of matrix metalloproteinases in human chondrocytes in vitro (340) Associated with glomerular hypertrophy in obese rats (339)
13-HpOTrE	Causes moderate and reversible depression in action potential parameters in rat cardiomyocytes (341)
13-oxo-OTrE	Induces glucose uptake and promotes adipocyte differentiation in murine model (342)
B. CYP oxylipins	
9,10-DiHODE	Lower in blood of hyperlipidemic vs. normolipidemic persons (120)
12,13-DiHODE	Lower in blood of hyperlipidemic vs. normolipidemic persons (120)

Table 2.6. Examples of EPA derived oxylipin functions

A. COX oxylipins	
15-deoxy-PGJ$_3$	Increases adiponectin secretion from murine adipocytes (343)
PGD$_3$	Lowers intraocular pressure in rabbit model (344)
	Decreases peripheral vascular resistance and increases cardiac output and heart rate in dogs (345)
	As potent as PGD$_2$ in modulating sympathetic nerve transmission in the eye but less effective in activating vagally mediated bradycardia in cat model (346)
	Inhibits human platelet aggregation with similar or greater activity than PGD$_2$ (187, 188)
PGE$_3$	Lowers intraocular pressure but caused mild conjunctival hyperemia in rabbit model (344)
	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 (347)
	Inhibits proliferation of human A549 cells and mouse melanoma B16 cells (348, 349)
	Less effective than PGE$_2$ in elevating plasma noradrenaline when administered intracerebroventricularly in rats (350)
	Less potent stimulator of cAMP production than PGE$_2$ in HEK293 human renal cells (148)
PGF$_{3\alpha}$	Less protective than PGF$_{2\alpha}$ on ethanol induced gastric mucosal injury in rat model (351)
PGI$_3$	Inhibits aggregation in human and rabbit platelets (352, 353)
	Promotes relaxation of bovine coronary arteries (353)
Δ^{12}-PGJ$_3$	Inhibits progression of leukemia in a mouse model (354)
TxA$_3$	Synthesized at a much lower rate than TxA$_2$ in human platelets (353)
	Elevates catecholamines when administered intracerebroventricularly as potently as TxA$_2$ in rats (350)
	Activates human platelet aggregation with potency comparable with TxA$_2$ (148)
B. LOX oxylipins	
5-HEPE	Enhances glucose-dependent insulin secretion in mouse MIN6 insulinoma cells and human NuTu80 intestinal carcinoma cells (355)
	Promotes bovine neutrophil chemotaxis in vitro, but less potently than 5-HETE (231)
5-HpETE	Inhibits human platelet aggregation, but less effectively than 12-HpEPE (232)
5-oxo-EPE	Stimulates migration of both human neutrophils and eosinophils at one tenth the activity of 5-oxo-ETE (153)
8-HEPE	Induces adipogenesis in mouse pre-adipocytes and glucose uptake in myoblasts via PPAR activation (69)
9-HEPE	Induces adipogenesis in mouse pre-adipocytes and glucose uptake in myoblasts via PPAR activation (69)
12-HEPE	Inhibits human platelet aggregation similarly to 12-HETE, but less effectively than 12-HPEPE or 12-HPETE (232)
12-HpEPE	Inhibits human platelet aggregation similarly to 12-HpETE, and more potently than 5- or 15-HpEPE (232, 248)
15-HEPE	Inhibits 5-LOX in rat basophilic leukemia cells (149)

	Inhibits cellular growth and AA metabolism in human prostatic adenocarcinoma cells (338)
15-HpEPE	Inhibits human platelet aggregation similarly to 15-HpETE, but less potently than 12-HpEPE (232)
	Inhibits glucosamine synthetase activity in rabbit gastric mucosa (356)
	Decreases rabbit renal prostaglandin synthesis (357)
	Inhibits AA metabolism in rabbit platelets (358)
LtA_5	Inhibits the formation of LtB_4 from LtA_4 by rat and human neutrophil LtA_4 hydrolase (359)
LtB_5	Less active than LtB_4 in aggregating rat and human neutrophils (150)
	Promotes chemotaxis of bovine or human neutrophils, but is much less potent than LtB_4 (231, 271)
LtC_5	Contracts guinea pig lung parenchymal strips and ileal tissues, with similar potency to LtC_4 (271)
	Inhibits the anaphylactic reaction in guinea pig isolated heart, with similar potency as LtC_4 (360)
	Contracts guinea pig ileum but less potently than LtC_4 (361)
LtD_5	Inhibited IL-1β-induced COX2 expression in human pulmonary microvascular endothelial cells (362)
	Stimulates volume regulation in murine Ehrlich ascites tumor cells (similar potency as LtD_4) (363)
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) (282)
	Induces superoxide anion generation from canine neutrophils and contraction of rat tail arteries (364)
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) (282)
	Induces superoxide anion generation from canine neutrophils (with similar activity to 4-series Lx) (364)
C. CYP oxylipins	
8,9-, 11,12-, 14,15-, 17,18-DiHETE	Inhibit human platelet aggregation, but with much less potency than parent EpETE (365)
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 (294)
	Inhibit human platelet aggregation and thromboxane synthesis, with similar potency to other EpETE and EDPE isomers, and greater potency than EpETrE isomers (365)
17,18-EpETE	Decreases human platelet aggregation (366)
	Relaxing effect on human bronchi arterial and airway smooth muscles (367)
	Anti-inflammatory effect in human lungs (368)
	Vasodilator in rat vascular smooth muscle cells (369)
18-HEPE	Inhibits macrophage mediated inflammation in cardiac fibroblasts in culture and prevents pressure overload–induced cardiac fibrosis and inflammation in mice (370)
	Decreases lipopolysaccharide-induced TNFα secretion in murine macrophage cell line (371)
RvE1	Reduces dermal inflammation, peritonitis, dendritic cell migration, and IL-12 production in

	an inflammatory mouse model (372)
	Reduces total leukocytes and PMN infiltration in murine peritonitis (373)
	Reduces hepatic fibrosis in murine model of infection (374)
	Promotes phagocyte removal during acute inflammation *in vitro* and *in vivo* (375)
RvE2	Stops zymogen-induced PMN leukocyte infiltration in murine peritonitis (376)
	Enhances phagocytosis and anti-inflammatory cytokine production in murine peritonitis (377)
	Inhibits human neutrophil infiltration and proinflammatory cytokines in an acute peritonitis (378)
RvE3	Inhibits neutrophil chemotaxis in vitro and reduces neutrophil numbers in zymosan-induced murine peritonitis in vivo (156)
	Blocks PMN infiltration in mouse model of peritonitis (379)

Table 2.7. Examples of DHA derived oxylipin functions

A. LOX oxylipins	
14,20-DiHDoHE	Inhibits PMN infiltration in the mouse peritonitis model (167)
14,21-DiHDoHE	Enhances wound healing in murine models (169, 170)
4-HDoHE	Inhibits endothelial cell proliferation and sprouting angiogenesis in mouse model of oxygen-induced retinopathy (380)
7-HDoHE	Activates PPARγ in transfected monkey kidney COS-7 cells (381)
13-HDoHE	Inhibits TNFα induced cytokine production in human microglial cells (93)
14-HDoHE	Inhibits human platelet aggregation (246)
17*S*-HDoHE	Vasodilates bovine coronary arterial smooth muscle cells (382)
	Reduces genotoxic and oxidative damage in murine hepatocyte cells and TNFα release by murine macrophages (381)
17*R*-HDoHE	Inhibits hyperalgesia in a rat model of adjuvant-induced arthritis (383)
	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (384)
	Inhibits TNFα induced cytokine production in human microglial cells (93)
17-HDoHE	Decreases lipopolysaccharide-induced TNFα secretion in murine macrophage cell line (385)
	Inhibits 5-LOX in rat basophilic leukemia cells (149)
17-HpDoHE	Displays cytotoxic potency in human neuroblastoma cells (386)
MaR1	Anti-inflammatory in a murine model of acute respiratory distress syndrome (387)
	Reduces inflammation- and chemotherapy-induced neuropathic pain in mice (388)
	Mitigates inflammatory effects of lipopolysaccharide-induced lung injury in mouse model (389)
PD1	Reduces genotoxic and oxidative damage in murine hepatocyte cells and TNFα release by murine macrophages (381)
	Promotes murine phagocyte removal during acute inflammation *in vitro* and *in vivo* (375)
	Decreases leukocyte accumulation in a mouse model of kidney injury (390)
	Protects human retinal pigment epithelial cells from apoptosis due to oxidative stress (391)
	Promotes mouse corneal epithelial cell wound healing (278)
PDX	Reduces inflammation in murine peritonitis and inhibits human microglial cell cytokine expression in vitro (158)
	Inhibits collagen, AA, and thromboxane induced human platelet aggregation (392)
	Inhibits PMN infiltration in mouse model of ischemic stroke (393)
	Decreases reactive oxygen species production and COX activity in human neutrophils (394)
	Improves insulin sensitivity by raising muscle IL-6 without affecting adipose tissue inflammation in murine model (395)
RvD1	Reduces reactivity and Ca^{2+} sensitivity in overactive human pulmonary artery smooth muscle cells (396)
	Improves bacterial clearance and survival of mice with cecal ligation and puncture induced sepsis (397)
RvD2	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (384)
	Improves bacterial clearance and survival of mice with cecal ligation and puncture induced

	sepsis (398)
	Inhibits inflammatory pain in mice (399)
	Mitigates neutrophil-mediated damage in mouse burn model (400)
RvD3	Reduces peritonitis and dermal inflammation in murine model (401)
RvD5	Enhances phagocyte containment of Escherichia coli in a mouse model (402)
AT-RvD1	Inhibits hyperalgesia in a rat model of adjuvant-induced arthritis (383)
	Anti-inflammatory effects in mouse model of dextran sulfate sodium-induced colitis (384)
AT-RvD3	Reduces murine peritonitis and dermal inflammation with activity similar to RvD_3 (401)
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 (365)
13,14-, 16,17-DiHDPE	Reduce pain associated with inflammation more potently than EpETrE and EpEPE (158)
13,14-DiHDPE	Markedly reduces potency to dilate porcine coronary arterioles compared to parent compound (403)
7,8-, 10,11-, 13,14-, 16,17-, 19,10-EpDPE	Dilates porcine coronary arterioles (403) Inhibits human platelet aggregation and thromboxane synthesis, with similar potency to other EpETE and EpDPE isomers, and greater potency than EpETrE isomers (365)
16,17-, 19,20-EpDPE	Inhibits Met-1 tumor angiogenesis and growth in mice (333)
19,20-EpDPE	Decreases human platelet aggregation (366)

2.2.5.1 N-6 PUFA Oxylipin Functions

COX oxylipins – Tables 2.1a, 2.3a, 2.4a. The most well known oxylipins are eicosanoids derived from the n-6 PUFA AA. COX derived prostanoids are involved in the regulation of blood pressure, reproduction, diuresis, blood platelet aggregation, modulation of the immune and nervous systems, gastric secretions, cancer, inflammation and the stimulation of smooth muscle contraction, among other effects, as reviewed (77, 79, 404-406). Within these COX metabolites there can be similar and differing effects on these functions. For example, PGI_2 is an anti-aggregatory factor for platelets (407), while TxA_2, serves as a pro-aggregatory factor (408). Another example is the vasodilatory effect of PGI_2 and PGE_2, and the vasoconstrictory effect of $PGF_{2\alpha}$ in some vascular beds (203, 409). PGE_2 also can have effects on thrombosis, which vary

depending on the receptor it interacts with. For example, PGE_2 can bind either the EP3 receptor, which makes PGE_2 a pro-thrombotic mediator, or EP4, which makes PGE_2 an anti-thrombotic mediator (410). Similarly, PGD_2 and its metabolites can be both pro-inflammatory and be involved in the resolution of inflammation (98). Compared to COX products formed from AA, those derived from DGLA are usually, but not always less active or produced less efficiently (411). For example, PGE_1 is less stimulatory of aortic smooth muscle cell proliferation than PGE_2 (412). The AdA metabolites, dihomo-PGE_2 and dihomo-PGI_2 also are inactive or much less active compared to their AA analogues with respect to their platelet aggregating activity and contractile properties in both vascular and nonvascular smooth muscle (144).

LOX oxylipins – Tables 2.1b, 2.2a, 2.3b. LOX products such as 5-, 12-, and 15-HETE derived from AA and secreted by epithelial cells and leukocytes are involved in many chronic diseases such as inflammation, obesity, cardiovascular disease, kidney disease and cancer (413-417). As is the case with COX metabolites, AA-derived LOX products can have effects that are both similar to and differing from each other, as well as from those derived via the COX and CYP pathways. For example, 12-HETE has been shown to have both pro- and anti-thrombotic effects (245, 418, 419), while TxA_2 is pro-thrombotic (408) and PGI_2 is anti-thrombotic (407). LOX derived HETE and their oxo-ETE metabolites appear to be primarily pro-inflammatory: for example 5-HETE has chemotactic roles in polymorphonuclear leukocytes (PMN) and rabbit alveolar macrophages (420, 421) and stimulates specific granule release from human neutrophils (227). Both 5-oxo-ETE and 12-oxo-ETE also can stimulate eosinophils and neutrophils, but appear to have less activity than their corresponding HETE (153, 233). 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 (422). Eoxins formed from 15-HpETE also have pro-inflammatory effects (95), 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 (261, 262).

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 (253, 254). 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 (81, 108, 423). 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 (424, 425).

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 (426-428), but there are also instances when HODE and their oxo-ODE metabolites are anti-inflammatory and anti-proliferative (242, 337, 429). While no functions for GLA oxylipins have been reported, DGLA oxylipins also tend to antagonize the analogous LOX derived AA oxylipins. For example, PGE_1 and 15-HETrE from DGLA have anti-proliferative effects, inhibit cancer cell growth and inhibit bleomycin-induced lung fibrosis (430-432), while 15-HETrE has anti-inflammatory effects in skin (337). Three-series leukotrienes derived from DGLA may also reduce inflammation and broncho-constriction due

to their relatively lower production compared to 4-series leukotrienes from AA and possibly lower bioactivity (433, 434).

CYP Oxylipins – Tables 2.1c, 2.2c, 2.4b. Oxylipins derived via the CYP pathway from AA include EpETrE and HETE, which have vascular, cardiac and renal functions (80, 435, 436). The effects of these oxylipins also are unique and can be opposing. For example, AA derived EpETrE formed via CYP epoxygenase have hypotensive effects, which is opposite to the hypertensive effects of 20-HETE formed via ω-hydroxylase activity (303, 437). In addition, 16-, 18- and 19-HETE, as well as 20-HETE metabolites (20-COOH-AA and 20-OH-PGE_2), also can promote vasodilation (300, 303, 438, 439). In some cases, the DiHETrE metabolites of EpETrE formed via sEH activity have less activity (298), but in other cases the DiHETrE have similar or even greater potency (286, 288). Interestingly, sEH inhibitors are currently being used to pharmacologically treat hypertension by prolonging the effects of the epoxy fatty acids on vasodilation (440), but polymorphisms in the CYP enzymes which produce EpETrE do not consistently correlate with effects on hypertension, as reviewed in (441). In addition, EpETrE also play roles in many other biological functions, such as insulin sensitivity (442), hyperalgesia (158) and tumor angiogenesis and metastasis (291, 297).

CYP oxylipins formed from LA appear to have similar effects to those derived from AA. For example, 9,10- and 12,13-EpOME derived from LA are produced by neutrophils and macrophages, mediating inflammatory effects (325, 443). 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 (444). Elevated EpOME also

has been related to extensive burns, respiratory syndrome and a systemic organ failure in burned skin of humans and lung (325).

2.2.5.2 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 (445). 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 (353, 446).

COX oxylipins – Table 2.6a. With respect to COX oxylipins, those derived from EPA are similar to DGLA oxylipins, generally being less potent or are produced less efficiently (353) 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 (445) and demonstrates less mitogenetic and inflammatory activity in fibroblasts and monocytes (347, 445, 447). TxA_3 compared to TxA_2 is produced less efficiently and was reported to have less vasoconstrictory and aggregatory activity (353), 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 (148). PGI_3 and PGI_2 also have similar vasodilatory and anti-aggregatory effects on platelets (353) and TxA_2 and TxA_3 have similar ability to elevate plasma catecholamines in rats, or to activate the TP receptor (148, 350, 353, 446).

LOX oxylipins – Tables 2.5a, 2.6b, 2.7a. LOX also metabolizes the n-3 PUFA, ALA to HOTrE, EPA to HEPE and DHA to HDoHE, oxylipins that also tend to have less inflammatory activity or to be anti-inflammatory. There is very little information on ALA

derived oxylipins, but recent findings indicate that 9,16-DiHOTrE has anti-inflammatory and anti-aggregatory effects by reducing prostaglandin production (147), and that 9- and 13-HOTrE are associated with reduced glomerular hypertrophy in obese rats (339). An earlier paper indicates that 13-HOTrE may have anti-inflammatory effects in chondrocytes (340), and a recent paper showed that 13-oxo-OTrE can stimulate glucose uptake and differentiation in adipocytes (342). EPA oxylipins have been much more investigated and are primarily anti-inflammatory; for example, 5-HpEPE can be metabolized to LtB_5, which has less activity, and also competes with LtB_4 and therefore reduces inflammation and broncho-constriction (448-450). 5-oxo-eicosapentaenoic acid (5-oxo-EPE) derived from 5-HEPE is 10-fold less potent in stimulating neutrophils compared with the AA oxylipin (5-oxo-ETE) derived from 5-HETE (153). 15-HEPE derived from EPA also exhibits anti-cancer effects. For example, in human prostatic adenocarcinoma cells 15-HEPE can inhibit cancer cell growth and inhibit production of AA oxylipins (338).

DHA also is metabolized via LOX, resulting in the production of HDoHE that also generally exhibit beneficial effects. For example, 4-HDoHE has been reported to inhibit proliferative retinopathy and retinal endothelial cell proliferation (380) and 14-HDoHE can antagonize platelet activation and smooth muscle constriction (246, 451). 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 (161, 452), or via other DiHDoHE which have similar protective effects, such as the wound healing properties of 14,21-DiHDoHE in mice (169) and inhibition of PMN infiltration in a mouse peritonitis model by 14,20-DiHDoHE (167). Similarly, 17-HDoHE inhibits 5-LOX in rat leukemia cells (149), reduces inflammation and oxidative damage in murine hepatocyte injury (381) and has anti-hyperalgesic properties in a

rat model of arthritis (383). 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 (163, 453, 454), while the effects of protectins vary by isomer – PDX has anti-aggregatory effects (392, 455) and can restore insulin sensitivity in obese mice (395), but PD1 does not exhibit these activities (395, 456). Both can inhibit influenza virus replication (457, 458), reduce inflammation and accelerate the resolution of inflammation (454), 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 (162).

CYP oxylipins – Tables 2.5b, 2.6c, 2.7b. 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 EpETrE in some vascular beds (403, 459). In addition, several CYP isoforms preferentially metabolize n-3 over n-6 PUFA, as reviewed in (171, 385). EpETE can also inhibit Ca^{2+} and isoproterenol induced contractility of neonatal cardiomyocytes, suggesting they have antiarrhythmic effects (154). EpDPE derived from DHA has anti-inflammatory, vasodilatory and anti-cancer effects, similar to EpETE (297, 366, 403). EpDPE also can inhibit angiogenesis and metastasis (297), unlike the AA derived EpETrE, which promote these functions (291). 18-HEPE derived from EPA via ω-hydroxylase also appears to have an anti-cancer role by down regulating pro-inflammatory and pro-proliferative factors (385), possibly via conversion to E-series resolvins. These resolvins have similar effects as the D-series resolvins,

markedly reducing PMN infiltration, decreasing pro-inflammatory cytokines, and enhancing the resolution of inflammation (423, 460, 461).

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

2.2.6 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 (462). 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 (162, 463). Recently, several reports have described the oxylipin profile in human blood (119, 464) and a small number of studies have examined the serum oxylipin profile of in response to fish oil supplementation in healthy individuals (465-468) as well as those who have asthma (469). These analyses and other studies that have increased dietary LA or ALA have

revealed that the type of dietary fat significantly alters oxylipin profiles (121, 339, 470, 471). 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 activities of oxylipins are needed in order to further our understanding of the physiological effects of the entire oxylipin profile. In addition, while some studies have compared the effects of oxylipin stereoisomers, much more knowledge in this area also is required. Differentiation between enzyme mediated and autooxidation products and their potential effects in biology will also be facilitated by these studies.

It is important to note that tissue PUFA composition cannot be used to reliably predict the oxylipin content of tissues, despite that this has routinely been done in the past literature. This was illustrated in a recent targeted lipidomic analysis of renal oxylipins in obese rats, which demonstrated that while the PUFA content generally reflected oxylipin content, there were notable discrepancies. For example, with 9-fold differences in the amounts of LA in the diets of these rats, the AA content of the renal phospholipid was the same, but the levels of several AA derived oxylipins were different (339). This has important implications for the current debate

surrounding the dietary recommendations for LA (472). Furthermore, this study indicated that PUFA conversion to oxylipins varies by as much as 10-fold between PUFA, with ALA being metabolized to oxylipins at a greater rate than LA, AA or EPA, for example. This may be due to differences in incorporation and release of phospholipid fatty acid, as well as differences in conversion to metabolites, which may be less, more or equally active. ALA also increased the level of oxylipins derived from EPA and DHA even though no EPA or DHA was present in the diets, demonstrating that PUFA also may mediate some of their effects via oxylipins derived from PUFA formed via elongation and desaturation of the shorter PUFA (339). Hence, there also is a need for kinetic analysis of oxylipin formation and turnover [also referred to as fluxolipidomics (473, 474)], which also will improve our understanding of the physiological effects of oxylipins in vivo. Comprehensive analyses that include the LA and ALA oxylipins in differing tissues in response to dietary interventions promises to yield significant novel information on the large numbers of these bioactive compounds

2.2.7 Transition to next section

As discussed in the above section, a large number of oxylipins are present in tissues and they regulate diverse physiological activities. In renal tissues, basic processes like glomerular filtration rate (GFR), salt-water balance etc. are regulated by these bioactive lipids. In diseased conditions, oxylipins influence proliferation, inflammation and fibrosis. Previous studies in rodent models of cystic kidney disease have shown alterations in the COX and LOX pathways that generate oxylipins in the renal tissue. Additionally, $cPLA_2$ levels were also affected in disease. Levels of COX derived oxylipins were higher in diseased kidneys compared to normal.

This suggests that oxylipins may play a crucial role in progression of PKD. The next chapter will review the literature on oxylipins in PKD.

2.3 Renal oxylipin levels are altered in polycystic kidney disease

2.3.1 Oxylipins in cystic kidney disease

Oxylipins control physiological processes in the normal kidney by regulating salt and water balance, renin release, vascular tone and glomerular filtration rate (GFR) (475-478). However, in diseased kidneys, oxylipins also contribute to kidney disease progression by mediating proliferatory, inflammatory, and fibrotic processes in response to kidney injury (479-482). In cystic kidney disease conditions, enzymes involved in the metabolism of oxylipins are affected. For example, cytosolic phospholipase A_2 ($cPLA_2$) is a major enzyme that releases PUFA from the membrane to be further converted to oxylipins. The level of $cPLA_2$ is elevated in rodent models of cystic kidney disease. In Han:SPRD-*Cy* rats and in *pcy* mice, which are non-orthologous models of PKD, membrane bound $cPLA_2$ levels were 34-131% higher in diseased renal tissues compared to normal (483). After release by cPLA2, PUFA is oxygenated by COX, LOX or CYP and further metabolised into numerous oxylipins (78, 480). Alterations in these enzymes and their products are discussed next.

2.3.2 Role of COX derived oxylipins from arachidonic acid in PKD

COX enzyme is normally expressed in both medulla and cortex of the kidney (484). Immunoblotting analyses of particulate kidney fractions revealed that expression of COX is elevated in both Han:SPRD-*Cy* rats and *pcy* mice (483). Enzyme activity assays also showed that in Han:SPRD-*Cy* rats, kidney disease was associated with elevated renal COX-1 and COX-2 enzyme activities (475, 485). Accordingly, pharmacological inhibition of COX reduced disease progression in this model by attenuation of renal fibrosis and reduction in renal cyst growth

(475). We have shown that COX derived arachidonic acid (AA) oxylipins $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGE_2, and TxB_2 were higher in Han:SPRD-*Cy* rats (10). In *pcy* mice, COX products from AA, including 6-keto-$PGF_{1\alpha}$, TxB_2 and PGE_2 are elevated in diseased kidneys as early as 30 days of age (18). Further, in another study, we have confirmed that COX derived AA oxylipins, PGD_2, PGE_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, 15-d-PGJ_2, TxB_2 and 12-HHTrE are elevated in the renal tissues of this model compared to normal mice (8). COX inhibition suppresses proliferation of human polycystic kidney disease epithelial cells and reduced disease in Han:SPRD-*Cy* rats (486). Results from these studies collectively signify the role of COX and COX metabolites in the progression of cystic kidney disease.

Oxylipins produced from AA via COX pathway have demonstrated roles in several mechanisms related to disease progression, such as stimulation of epithelial cell proliferation and trans-epithelial fluid secretion (487-489). Renal cyst expansion in cystic kidney disease is highly influenced by these two processes (2, 490), and are stimulated by cAMP (489, 491). COX metabolites from AA such as PGI_2, PGD_2, and PGE_2 regulate the formation of cAMP (489). Additionally, cellular cAMP levels may also be elevated by PGs by inhibition of cAMP phosphodiesterase that hydrolyses cAMP (492). Accordingly, augmentation of cell proliferation, fluid secretion and cyst formation by COX metabolites PGE_2, PGI_2, and TxB_2 have been reported in renal epithelial cell models from humans and rodents (487, 493, 494). Also, AA derived prostanoids (PGE_2, PGI_2, TxA_2) are higher in diseased Han:SPRD-*Cy* rats compared to normal rats (485). Although these studies provide compelling evidence to the role of oxylipins formed from AA via COX pathway in the disease progression, the number of oxylipins studied is limited. There is a large number of novel AA-COX oxylipins discovered in the last decade due

to advances in lipidomic methods. It is essential that the role of these oxylipins be explored further.

2.3.3 Role of n-3 and n-6 PUFA derived oxylipins from other pathways

While most studies have focused on AA metabolites of COX, oxylipins from other n-6 PUFA and n-3 PUFA derived via COX, LOX and CYP pathways may also have additional effects. In general, oxylipins from n-3 PUFA oppose the actions of those derived from n-6 PUFA. There are indications that COX, LOX and CYP products from n-3 and n-6 PUFA (other than AA) are altered in PKD (8, 10). For example, in *pcy* mice COX derived EPA oxylipins were higher in diseased kidneys compared to normal (8). In contrast, LOX derived LA oxylipin 9-HODE was found to be lower in kidneys of Han:SPRD-*Cy* rats (10) where as several LOX oxylipins formed from LA, DGLA, ALA, AA, EPA and DHA were lower in *pcy* mouse kidneys (8). The functions of LOX oxylipins are comparatively less studied, but those formed from AA and LA are generally considered pro-inflammatory while those derived from n-3 PUFA are biologically less active or are anti-inflammatory (428, 495, 496). Studies examining the levels of CYP derived oxylipins are rare. We recently reported alterations in CYP pathway in *pcy* mouse (8). In this study, total and individual CYP oxylipins from LA (TriHOME, DiHOME), AA (DiHETrE, HETE), ALA (EpODE), EPA (HEPE, DiHDPA) and DHA (HDoHE) were lower in renal tissues of *pcy* compared to normal mice (8). A recent report on patients with renal cystic disease showed lower levels of plasma EpETE, a CYP oxylipin (497). 20-HETE formed via CYP promotes cyst formation in the Balb/C polycystic kidney (BPK) mouse model of autosomal recessive PKD both in vitro and in vivo (498). Inhibition of CYP has

been shown to reduce disease progression in this model and in PCK rats (498, 499). Additionally, CYP derived oxylipins has proven cardiovascular and renal benefits, including vasodilatory, anti-inflammatory, anti-fibrotic and anti-apoptopic effects (500-502). Therefore, it is reasonable to speculate that lower levels of CYP derived oxylipins observed in PKD may have a role in disease progression.

Thus, while the role of oxylipins produced from AA via the COX pathway in the progression of PKD is relatively better understood, the effects of those derived from other n-6 PUFA and any n-3 PUFA needs further research. In the past, a limitation was that the radiometric and enzyme linked immune sorbent assay was not useful in analysing 100s of molecules at a time. Recent advancements in targeted lipidomics based approaches have enabled the analysis of large numbers of oxylipins present in tissues simultaneously. Therefore, it is imperative that the presence and levels of oxylipins in this disease be elucidated so as to potentially facilitate therapeutic interventions.

2.3.4 Transition to next section

As discussed above, it is clear that oxylipins are associated with progression of PKD. Oxylipins derived through the COX pathway from AA appear to be particularly important. Dietary n-3 fatty acids are effective in reducing the tissue levels of n-6 PUFA. Reduction in disease has been observed consistently in PKD with flax oil feeding, but the results from fish oil feeding are inconclusive. Studies with dietary supplementation with these oils also showed reductions in AA derived oxylipins and increases in n-3 fatty acid derived oxylipins. Soy protein is another dietary element that has beneficial effects on PKD, and is also effective in reducing COX levels. The next section will discuss the effect of dietary flax oil, fish oil and soy protein on disease and renal oxylipin levels.

2.4 Dietary interventions in PKD improves the disease and modulates oxylipin levels

2.4.1 Dietary therapy in PKD

Despite having a reasonable understanding of the genetic causes, proteins involved and the mechanism of disease progression in PKD, a curative treatment option has not yet emerged. Although several pharmacological interventions such as vasopressin receptor antagonists have shown potential, they either come with undesirable side effects (503) or fail to delay the need of kidney transplant (504). This has led to great interest in alternative therapies to slow the disease progression, including dietary interventions. PKD associations in various countries provide nutritional advice to PKD patients (11-13). Evidence for these dietary recommendations come from the benefits seen in non-orthologous animal models of PKD, including Han:SPRD-*Cy* rats and *pcy* mice (14, 15). PKD organizations in Canada and the UK recommend oils containing n-3 PUFA and soy protein for PKD patients, along with other strategies such as reducing protein and sodium intake and increasing antioxidant and fiber rich foods (11, 12). In Han:SPRD-*Cy* rats and *pcy* mice, dietary soy protein compared to casein yielded lower kidney size and water content, reduced cyst growth and fibrosis (4, 5, 7, 505-508). Dietary flax oil rich in ALA also has been consistently shown to reduce renal size, water content, cyst growth and fibrosis (6, 8, 9, 509). Fish oils rich in EPA and DHA have shown beneficial effects in some studies (10, 510) but not always (9, 511, 512). Studies that examined enzymes involved in the oxylipin metabolism have found that in addition to improvements in disease, dietary soy protein, flax and fish oil also affects $cPLA_2$ and COX expression (10, 513). Consequently, disease associated alterations in renal oxylipin levels were reversed in some cases with these dietary treatments (8, 10). Therefore, dietary interventions involving soy protein and n-3 rich oils holds considerable potential as a

lifestyle modification to slow down the disease to improve the quality of life of patients and to delay the need for renal replacement therapy.

2.4.2 Effect of dietary flax oil on disease and renal oxylipins

There have been several studies in the last decade that investigated the effect of flax oil on PKD. Recently, our lab has reported that feeding flax oil to *pcy* mice results in reduction in renal fibrosis and improvement in kidney function (8). In these animals, disease was associated with elevated levels of several AA derived oxylipins and reduced levels of ALA and DHA oxylipins. Flax oil feeding not only lowered the AA acid derived oxylipins, but also increased the ALA, EPA and DHA derived oxylipins. Increase in DHA oxylipin was attained without any significant increase in tissue DHA level (8). It is not clear whether the benefits in disease were brought about by the reduction in n-6 oxylipins or by the elevation of n-3 oxylipins or both. In another study, Sankaran et al reported that flax oil consumption mitigated the detrimental effects of high fat diet on renal fibrosis in young *pcy* mice (9), but the oxylipin levels were not reported.

Ogborn et al demonstrated the ability of flax oil to diminish renal cyst area and fibrosis, macrophage infiltration and epithelial proliferation in Han:SPRD-*Cy* rats (509). Similar reductions in cyst area was observed in male and female Han:SPRD-*Cy* rats with flax seed consumption, and reduction in fibrosis, inflammation and epithelial proliferation was seen in females (6). AA derived oxylipin PGE_2, which is shown to accelerate proliferation and inflammation was lowered by flax oil in these animals (6).

It is evident from the above studies that flax oil is beneficial in cystic disease. It appears from the limited oxylipin results from some of the above studies that the benefits of flax oil may be mediated via modulation of oxylipin levels. This is an important aspect to be further studied,

since flax oil feeding has been demonstrated to reduce the level of pro-inflammatory and proliferatory oxylipins produced from AA and those produced from LA in rat renal tissues (121). In older humans, four weeks of consumption of flax seed containing 6g ALA per day lowered the levels of AA derived 5-HETE and LA derived 9,10,13-TriHOME and 9,12,13-TriHOME in plasma (514). Flax oil feeding also results in elevated ALA and EPA derived oxylipins (121). Although less studied, some oxylipins from ALA such as HOTrEs are shown to have anti-inflammatory properties in-vitro (515). Oxylipins from EPA do have the ability to oppose the detrimental effects of AA derived oxylipins (516). Therefore, the role of flax oil modulated oxylipin levels in the reduction of the disease needs to be better understood.

2.4.3 Effect of dietary fish oil on disease and renal oxylipins

Studies with fish oil intervention on PKD has yielded conflicting results in the past with beneficial effects reported in Han:SPRD-*Cy* rats (10, 510), but not always in *pcy* mouse (9, 511, 512). We recently studied the effect of fish oil on oxylipin levels and disease in Han:SPRD-*Cy* rats(10). Fish oil treatment reversed the elevated levels of AA derived oxylipins formed via the COX pathway ($PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGE_2, and TxB_2) in diseased kidneys, but enhanced disease associated reduction in oxylipins from the LOX pathway (5- and 15-HETE and 9-HODE) (10). Differences in LOX products were not parallel to disease. Additionally, oxylipins derived from n-3 PUFA (TxB_3, $PGF_{3\alpha}$, PGD_3 and PGE_3) were elevated in diseased kidneys by fish oil treatment. These changes were accompanied by moderate reduction in disease in these animals (9). An earlier study on Han:SPRD-*Cy* rats on high fat diet have reported significantly

lower kidney weights, kidney water content, cyst volumes and renal fibrosis with fish oil treatment, but did not measure oxylipin levels (510).

Sankaran et al studied the effect of DHA rich algal oil in male and female *pcy* mice (9). In young *pcy* mice, algal oil treatment resulted in higher kidney weights, serum urea nitrogen, and cyst volumes (9). Two other studies on *pcy* mice also reported lack of effect dietary fish oil on disease progression (511, 512). However, an earlier study by Yamaguchi et al reported lower mean kidney weights and tubular dilatation in both male and female *pcy* mice, and lower cyst area in male *pcy* mice, with fish oil treatment (517). None of these studies on *pcy* mice measured the effect of fish oil on renal oxylipin levels.

Therefore, the effect of fish oil rich in long chain n-3 PUFA on PKD remains unclear. However, our recent study on Han:SPRD-*Cy* rats (10) provide indications that fish oil modulates disease associated alterations in some of the oxylipins diseased kidneys. This calls for further exploration of role of fish oil in regulating oxylipins levels in PKD.

2.4.4 Effect of dietary soy protein on disease and renal oxylipins

Dietary soy protein has consistently demonstrated protective effects in spontaneous models of cystic kidney disease including Han:SPRD-*Cy* rats (4, 5, 7, 10, 505) and *pcy* mice (506), as well as other renal disease (518, 519). Although the effects of soy protein on tissue fatty acid levels are minimal, there are indications that renal oxylipins alterations are associated with the beneficial effects of soy protein (10). In a recent study in Han:SPRD-*Cy* rats, soy protein was effective in ameliorating disease progression, and the changes in disease were parallel to soy protein mediated reversal of disease associated alterations in renal oxylipins (10). In the renal cortex of these animals, soy protein feeding restored the elevated levels of AA

derived 6-keto-$PGF_{1\alpha}$, PGE_2, and TxB_2 to normal levels. Another study by Peng et al on the same model showed a reversal of disease associated elevation of 6-keto-$PGF_{1\alpha}$, PGE_2 and TxB_2 in the renal tissues (513). In a study on healthy individuals, a soy based diet for 3-weeks lowered glomerular filtration rate (GFR) and this was associated with reductions in PGI_2 levels (520). The results from these studies suggest an ability of soy protein to reduce n-6 PUFA derived oxylipins. It is also demonstrated that dietary soy protein affects the levels and activity of of Δ-6 desaturase, an enzyme that converts LA to longer chain PUFA (521, 522). However, the effect this has on the renal oxylipin profile remains to be investigated. Also it is not clear whether the reversal of disease related oxylipin changes in response to soy protein feeding is a direct effect or an indirect consequence of soy protein affecting disease via a different mechanism. It appears that the mechanism of soy protein mediated changes in renal oxylipins is different from those of n-3 PUFA. The effect of dietary protein on oxylipins is immediate (523) whereas dietary PUFA must be incorporated to tissue phospholipids before oxylipin synthesis is affected, which could take up to 3 weeks (524, 525). If dietary soy protein and n-3 PUFA are affecting disease via different mechanisms, it could mean that an intervention with a combination of soy protein and n-3 PUFA would yield added benefits. Thus, understanding of oxylipin modulations by dietary soy protein offers a novel arena of research into disease management including potential for pharmacological interventions.

2.4.5 Orthologous vs non-orthologous models

Most of the studies examining the effects of nutritional interventions in PKD have been performed on spontaneous non-orthologous models, the Han:SPRD-*Cy* rat and the *pcy* mouse. While phenotypic expression of disease is similar to humans in these models, the genetic causes

are different. Han:SPRD-*Cy* rat has a mutated Anks6 (formally called Pkdr1) gene (526) and the *pcy* mouse harbors the Nphp3 mutation (527). These models are suitable models of human NPHP than human PKD (527, 528). Human ADPKD is caused by mutations in *PKD1* and *PKD2*, genes for polycystin 1 and 2 respectively (30) and ARPKD is caused by a mutation in the polycystic kidney and hepatic disease-1 (*PKHD1*) gene, which codes for polyductin/fibrocystin (529). A short-term study in human PKD failed to replicate the beneficial effects of n-3 PUFA seen in these non-orthologous models (17). Recently, Maditz et al used PCK rats, which is an orthologous model of ARPKD, for examining the effect of dietary soy protein and fish oil, but found no beneficial effects (16). This raises the questions whether dietary recommendations should be formed based on results from non-orthologous models where the underlying generic cause is different than humans. Moreover, orthologous models of ADPKD have only been available recently and nutritional studies have not been performed on them. Therefore, since no model replicates the human form of disease completely, it is necessary to replicate results in multiple models before conclusions can be drawn.

2.4.6 Effect of sex on disease and oxylipins

The majority of the studies in PKD using soy protein and n-3 PUFA have been performed on male animals despite the existence of gender differences (22-24). In Han:SPRD-*Cy* rats females have slower progression and males display higher degree of disease related renal abnormalities (530). With respect to nutritional intervention, recent studies on female PCK rats have not shown any benefit of soy protein or fish oil (16).

Oxylipin metabolism also appears to be influenced by gender. Differences have been reported between males and females in enzymes that metabolize AA and AA derived oxylipins

(531, 532). Consequently, higher levels of PGE_2 and TxB_2 were secreted in the urine of diseased female rats compared to males (533). In plasma, higher levels of PGE_2 and PGI_2 were seen in female than male in rats (534). In renal tissues, female rats had higher PGE_2 synthase and COX-2 protein expression (531). Other factors such as effect of female hormones on enzymes involved in oxylipin metabolism, such as 15-hydroxyprostaglandin dehydrogenase (532, 535) and prostaglandin 9-ketoreductase (536) might also play a role. Hatano et al recently discovered that sex hormones influence a PG-specific transporter responsible for PG clearance in rat renal tissues (537). Females had lower expression of these transporters and thus higher levels of AA derived PGE_2 in the kidney. These results point to the possibility of sex specific differences in the effects of dietary intervention on disease and oxylipin production, and needs to be further studied. Also a detailed characterization of renal oxylipins by sex has never been reported.

Chapter 3

3.1 Hypothesis

Based on data from non-orthologous models, it is hypothesised that in orthologous models, dietary flax oil, fish oil and soy protein will reduce disease progression. It is also hypothesised that PKD will result in disease specific alterations in renal oxylipins produced from n-3 and n-6 PUFA generated through COX, LOX and CYP pathways. It is further hypothesised that dietary interventions with soy protein and oils rich in n-3 PUFA will reverse the disease specific alterations in oxylipin. Such reversal will be associated with beneficial effects on the progression of polycystic kidney disease in these models.

3.2 Objectives

The main purpose of this research was to investigate the effects of dietary soy protein, flax oil and fish oil on disease progression and the renal oxylipin profile in polycystic kidney disease using orthologous models. The current research will also examine if there exist any sex specific changes in either disease or oxylipins. Therefore, the above hypotheses were tested through the following objectives:

(i) Examine whether dietary intervention using soy protein, flax oil or fish oil improves renal disease markers in orthologous models of PKD.

(ii) Compare normal and diseased animals to determine if there are disease specific alterations in the renal oxylipins produced via COX, LOX and CYP pathways.

(iii) Examine the effect of dietary treatments on the oxylipins to determine if disease specific alterations are reversed by diet.

(iv) Determine whether sex of the animal influences disease progression, oxylipin alterations or effect of dietary treatments.

Chapter 4

4. Lack of benefit of early intervention with dietary flax and fish oil and soy protein in orthologous rodent models of human hereditary polycystic kidney disease

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4.1 Abstract

Rationale for dietary advice in polycystic kidney disease (PKD) is based in part on animal studies that have examined non-orthologous models with progressive development of cystic disease. Since no model completely mimics human PKD, the purpose of the current studies was to examine the effects of dietary soy protein (compared to casein) or oils enriched in omega-3 fatty acids (fish or flax oil compared to soy oil) on early disease progression in two orthologous models of PKD. The models studied were *Pkd2*$^{WS25/-}$ mice as a model of autosomal dominant PKD, and PCK rats as a model of autosomal recessive PKD. After 13 weeks of feeding, dietary fish (but not flax) oil resulted in larger kidneys and greater kidney water content in female *Pkd2*$^{WS25/-}$ compared to control mice. After 12 weeks of feeding male PCK compared to control rats, both fish and flax compared to soy oil resulted in enlarged kidneys and livers, greater kidney water content and higher kidney cyst area in diseased rats. Dietary soy protein compared to casein had no effects in *Pkd2*$^{WS25/-}$ compared to control mice. In PCK rats, kidney and liver histology were not improved, but lower proteinuria and higher urine pH suggest that soy protein could be beneficial in the long term. Therefore, in contrast to studies in non-orthologous models during the progressive development phase, these studies in orthologous PKD models do not support dietary advice to increase soy protein or oils enriched in omega-3 oils in early PKD.

4.2 Introduction

Hereditary polycystic kidney disease (PKD) is characterized by countless renal cysts and often also displays significant liver cysts. The two major types of PKD are autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). Approximately 80-85% of ADPKD is caused by mutations in *PKD1,* the gene for polycystin 1, and the remaining 15-20% of cases are caused by mutations in *PKD2,* which codes for polycystin 2 (30). ARPKD is much less common but is a more severe form of disease that primarily affects the pediatric population. ARPKD is caused by a mutation in the polycystic kidney and hepatic disease-1 (*PKHD1*) gene, which codes for polyductin/fibrocystin (529)

Despite the promise of pharmacological treatments such as vasopressin receptor antagonists, the presence of undesirable side effects (538) and the lack of efficacy of current treatments in delaying the need for renal replacement therapy (504), has led to much interest in alternative treatments such as dietary therapy. This is evidenced by ADPKD diet studies currently in progress (539, 540), as well as nutritional advice on several national PKD association web pages addressing nutrition questions (11-13). In their dietary advice, PKD Foundations in both Canada and the US cite animal studies as evidence of the potential effectiveness of dietary soy and plant proteins. Dietary soy protein has been particularly effective in several spontaneous rodent models of renal cystic diseases such as the Han:SPRD-*Cy* rat with the mutated *Anks6* (formally called *Pkdr1*) gene and the *pcy*/*pcy* (*pcy*) mouse that harbors the *pcy* mutation (4, 5, 7, 505-508). In these models, renal cyst disease develops progressively and soy protein feeding resulted in lower kidney size and water content, along with reduced cyst growth and fibrosis, when replacing casein as the protein source in the standard AIN93 laboratory rodent diet (541).

The PKD Foundations in Canada and the US also advise patients to consume omega-3 fatty acids, again based on animal model data. Indeed, dietary flax oil (enriched in alpha-linolenic acid) reduces kidney size, water content, cyst growth and fibrosis in both the Han:SPRD-*Cy* rat and the *pcy* mouse (6, 8, 9, 509, 542). However, fish oil (enriched in EPA and DHA) appears to have beneficial effects in the Han:SPRD-*Cy* rat (10, 510), but not always in the *pcy* mouse (9, 511, 517, 543).

In both dietary soy protein and omega-3 oil interventions, male animals have been used, as they typically display greater disease progression. However, recent studies in the female PCK rat model of ARPKD have suggested that dietary soy protein or fish oil may not be effective in this orthologous model of ARPKD (16) Further, the effects of dietary soy protein or omega-3 oils in orthologous models of ADPKD have not yet been determined in either males or females, nor have male PCK rats been tested with these dietary treatments. Therefore, the effects of soy protein compared to casein and flax or fish oil compared to soy oil were examined in the early stages of disease in males and females of an orthologous mouse model of ADPKD (*Pkd2*$^{ws25/-}$ mice) and in the male PCK rat model of ARPKD.

4.3 **Materials and Methods**

All animal procedures were approved by the University of Manitoba Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care.

4.3.1 *Pkd2*$^{ws25/-}$ mice

Pkd2$^{ws25/ws25}$ and *Pkd2*$^{+/-}$ breeders were obtained from Dr. Stefen Somlo at Yale University (New Haven, CT, U.S.A.) (544). These genotypes were crossed to produce mice

with diseased (*Pkd2*$^{ws25/-}$) or normal (*Pkd2*$^{ws25/+}$) phenotypes. All mice were given diets based on the American Institute of Nutrition (AIN) 93G standard diet for laboratory rodents (541), which has casein as the standard protein source and soy oil as the standard oil. The experimental diets contained either an equivalent amount of soy protein that replaced the casein, or either flax oil or fish oil that replaced 80% of the soy oil, as shown in Table 4.1 and detailed in previous studies of non-orthologous models of PKD (9, 508). All oils and diet ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA). The oils contained 0.02% tBHQ to prevent oxidation and diet ingredients were stored at 4˚C. Diet was freshly prepared twice per month and stored in sealed containers at -20˚C until feeding. Routine examination of texture, odor, and color indicated that the oils were not oxidized. Mice were housed singly in cages with plastic enrichment domes in a temperature- and humidity-controlled environment with a 12 hour day/night cycle and were given free access to water and diet.

The feeding period was for 13 weeks, from 3 to 16 weeks of age, and feed and water disappearance were determined during week 6 of feeding to estimate feed and water intakes, respectively. Mice were monitored daily and no mice became ill or died. Mice were anesthetized to surgical plane using isofluorane and euthanized via exsanguination. Normal and diseased mice were identified by the absence or presence of renal cysts. Body, kidney and liver weights were recorded before placing the left kidney and a portion of the liver in 10% formalin for 24h, followed by transfer to PBS at 4˚C until further processing. The right kidney and another portion of the liver were snap frozen in liquid nitrogen, and lyophilized to determine tissue water content.

Formalin fixed kidneys and livers were embedded in paraffin, sectioned at 5 μm and tissue sections were stained with Masson's trichrome to measure cyst and fibrosis area as

previously described (9, 510). A Nikon D600 FX DSLR camera equipped with a 60mm F2.8 Macro lens (Nikon Corporation, Mississauga, Canada) was used to capture images of backlit whole kidney sections. Macro rings were used between the camera body and lens to achieve 2.5X magnification. This allowed clear identification of open spaces from complete coverage of the kidney or liver sections in each picture. Quantitative analysis of cyst area of the whole kidney section and the sample liver section was performed using Image Pro software (Media Cybernetics, Silverspring, MO), after coloring in the white areas of tubular lumen spaces to eliminate these from measurement. In addition to cyst area, the blue areas in stained sections were used to examine fibrosis by densitometry as previously described (9, 510).

4.3.2 PCK rats

Weanling male PCK rats were purchased from a commercial breeder (Charles River, QC, Canada) and normal and diseased rats were provided the same diets and housing conditions as described for study 1. PCK rats were fed these diets for 12 weeks, from 4 to 16 weeks of age, and feed and water disappearance determined during week 10 of the feeding period using metabolic cages. Rats were monitored daily and one rat in the casein fish oil group was euthanized in week 2 due to poor overall condition and was found to have an enlarged heart upon autopsy. A second rat in the casein fish oil group was terminated in week 12 due to excessive weight loss and poor condition, and was found to have enlarged kidneys, liver and spleen upon autopsy. Rats were anesthetized as described for mice. At termination, tissues were processed as described above for *Pkd2*$^{ws25/-}$ tissues, with the exception of cyst area measurements, for which a Nikon D90 DX DSLR camera was used. Tubular lumen spaces could not be accurately differentiated from cysts in these sections, so white spaces from both the cysts and tubular lumen spaces in these sections were quantified together.

Table 4.1 Details of experimental diets based on the AIN-93G diet for laboratory rodents (541).

Protein source	Casein			Soy Protein		
Oil source	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil
Ingredient	*g/kg diet*					
Casein (87% protein)	200	200	200	-	-	-
Soy protein (92% protein)	-	-	-	*189*	*189*	*189*
Soybean oil	70	*14*	*14*	70	*14*	*14*
Fish oil	-		*56*	-	-	*56*
Flax oil	-	*56*	-	-	*56*	-
Cornstarch	397.5	397.5	397.5	*408.5*	*408.5*	*408.5*
Dextrinized cornstarch	132	132	132	132	132	132
Sucrose	100	100	100	100	100	100
Fibre (cellulose)	50	50	50	50	50	50
Mineral mix (AIN93G)[1]	35	35	35	35	35	35
Vitamin mix (AIN93G)[1]	10	10	10	10	10	10
L-cystine	3	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Tert-butylhydroquinone[2]	0.014	0.014	0.014	0.014	0.014	0.014

[1]Details of the mineral and vitamin mix are found in(541).
[2]Antioxidant added to the oils.
The shaded numbers are those that are modified from the control diet (casein protein, soy oil) to create the experimental diets.

Serum and urinary creatinine were measured using the Jaffe reaction as modified by Heinegard & Tiderstrom and adapted for micro assay (9). Creatinine clearance was calculated using urine volume and urine creatinine from week 10 of the feeding period and serum creatinine from termination. Urine pH was measured immediately after 24 hour urine collection and urine protein was determined using the Bradford protein assay method with bovine serum albumin as a standard (545). Blood pressure was measured in conscious rats at week 12 of feeding period using a multichannel blood pressure system with a tail-cuff sphygmomanometer (Coda 6 System, Kent Scientific, Torrington, Conn), as described (546).

4.3.3 Statistical Analyses

To first determine effects of disease and sex, only those provided soy oil (control oil) were compared. In *Pkd2*$^{WS25/-}$ and *Pkd2*$^{WS25/+}$ mice, 3-way ANOVA (sex x disease x protein) revealed that there were no protein effects on disease, so mice given soy protein and casein were combined for analyses by 2-way (disease x sex) ANOVA. For PCK rats, only males were used, so disease effects in the soy oil fed rats were tested using t-tests.

Dietary effects were tested in *Pkd2*$^{WS25/-}$ animals only, and 3-way ANOVA (sex x oil x protein) again revealed that protein had no effect on any parameters. Therefore, mice given soy protein and casein were combined and a 2-way ANOVA (oil x sex) was performed. For PCK rats 2-way ANOVA (oil x protein) was used, as only males were used for this study.

All ANOVA were performed using the GLM procedure of SAS (SAS, version 9.3, Cary, NC) followed by Duncan's Multiple Range test to delineate significant oil or interaction effects. Normality of the data was assessed using the Shapiro–Wilk's test, and non-normal data was normalized by log transformation where possible. If normality was not achieved, data were

analyzed using the Kruskal–Wallis test. Statistical significance for main and interaction effects was set at $P < 0.05$. All data are presented as mean±SE.

4.4 Results

4.4.1 *Pkd2*$^{WS25/-}$ mice

At the end of the feeding period, the area comprising both cysts and tubular lumen made up ~15-20% of the kidney section areas in *Pkd2*$^{WS25/-}$ mice (Figure 4.1A-D). On the other hand, livers displayed fewer and smaller cysts, with 42% of mice exhibiting no cysts at all, and none displaying significant fibrosis (Figure 4.2A-D). As well, 3-way (sex x disease x protein) ANOVA of the soy oil fed mice revealed that there was no protein effect on any of the parameters measured, indicating no benefit of soy protein compared to the casein protein. The protein groups were therefore combined, and in mice provided the soy oil diets, cyst development resulted in higher kidney weights and water content in diseased compared to normal mice (Table 4.2). Consistent with the small and sporadic liver cysts observed, liver weights were not elevated in diseased compared to normal mice. Body and tissue weights were higher in males compared to females, but there were no sex differences in any other parameters (Table 4.2).

With respect to dietary oil effects, fish oil effects on renal disease were only observed in female mice: females given fish oil compared to either soy or flax oil had higher kidney weights and kidney water content. However, there were neither significant dietary oil effects on renal cyst area, nor on any liver parameters. All parameters in flax oil fed mice were similar to soy oil fed mice, with the exception of higher kidney weights in flax oil fed male mice. There were no dietary effects on feed intake, water intake or body weight (Table 4.3 and figure 4.3 and 4.4).

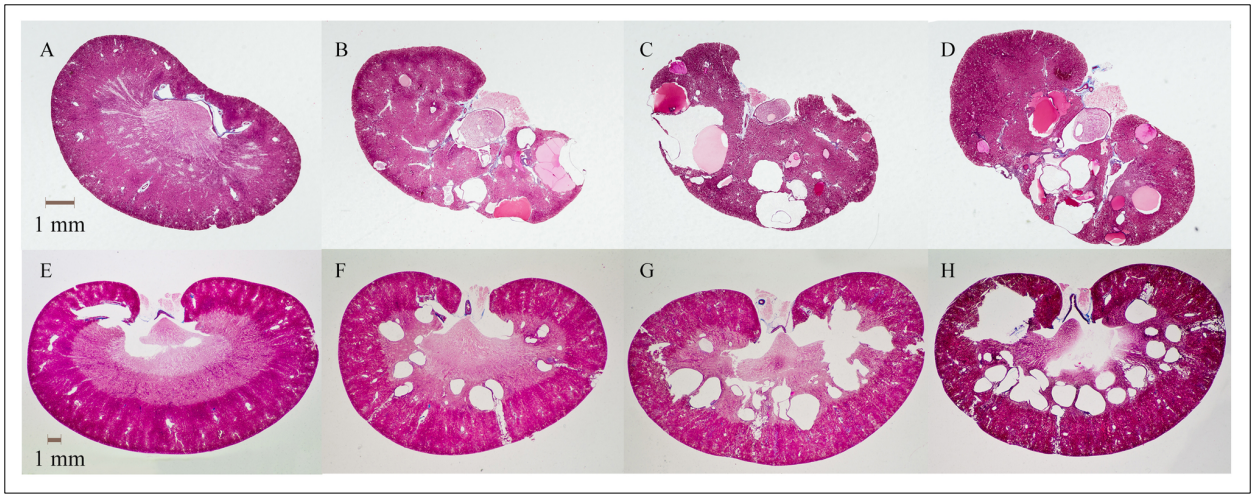

Figure 4.1: **Kidney sections.** Sections A-D are from Pkd2 mice and E-F are from PCK rats as follows: (A) *Pkd2*$^{WS25/+}$ (normal) and (B)*Pkd2*$^{WS25/-}$ (diseased) mice provided soy oil, *Pkd2*$^{WS25/-}$ (diseased) mice provided (C) flax oil or (D) fish oil, (E) normal and (F) PCK rats provided soy oil, PCK rats provided (G) flax oil or (H) fish oil. Scale bar = 1 mm.

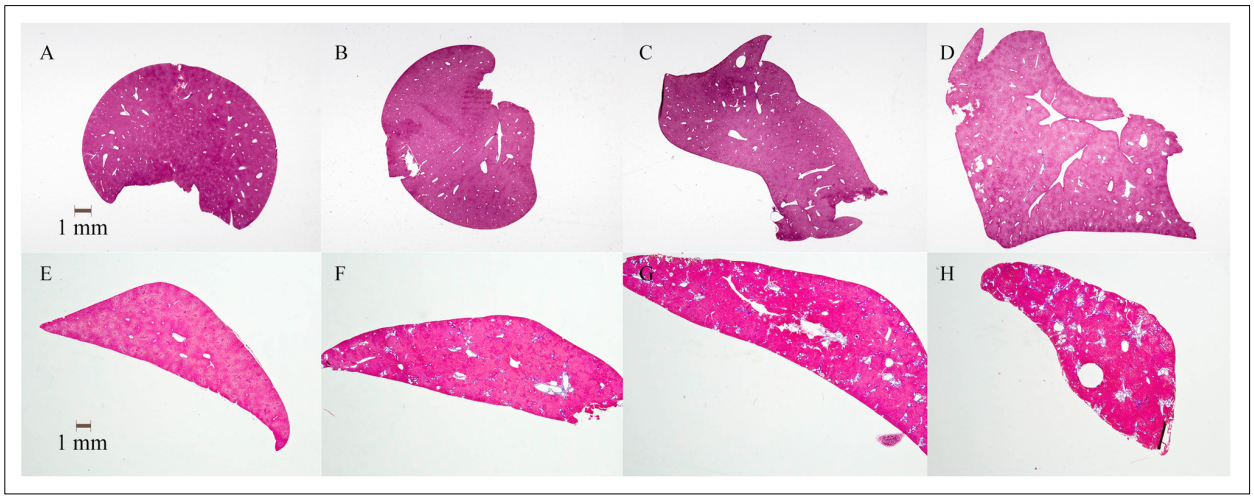

Figure 4.2: **Liver sections.** Sections A-D are from Pkd2 mice and E-F are from PCK rats as follows: (A) *Pkd2*$^{WS25/+}$ (normal) and (B)*Pkd2*$^{WS25/-}$ (diseased) mice provided soy oil, *Pkd2*$^{WS25/-}$ (diseased) mice provided (C) flax oil or (D) fish oil, (E) normal and (F) PCK rats provided soy oil, PCK rats provided (G) flax oil or (H) fish oil. Scale bar = 1 mm.

Table 4.2. Disease and sex effects in *Pkd2*$^{WS25/-}$ and *Pkd2*$^{WS25/+}$ mice

	Pkd2$^{WS25/+}$		*Pkd2*$^{WS25/-}$			
	Male	Female	Male	Female	P<0.05	
Body weight (g)	26.2±2.2	19.5±0.6	27.1±1.7	19.3±1.0		S
Kidney						
weight (g)	0.28±0.02	0.21±0.01	0.37±0.04	0.27±0.03	D	S
weight / body weight (g/100g)	1.05±0.04	1.11±0.04	1.37±0.01	1.40±0.12	D	
water content (%)	72.2±0.6	71.5±1.0	75.4±1.8	74.8±1.1	D	
cyst area / section (pixels x10^3)			468±94	419±99		
cyst area / kidney area (%)	-	-	16.6±5.5	20.9±8.3		
Liver						
weight (g)	1.10±0.12	0.77±0.03	0.98±0.15	0.77±0.06		S
weight / body weight (g/100g)	4.06±0.13	3.98±0.06	4.25±0.09	3.95±0.13		
water content (%)	66.9±0.5	66.7±0.7	66.7±0.6	67.2±2.0		
cyst area / liver area (%)	-	-	0.24±0.20	0.07±0.03		
Feed intake (g/24h)	3.3±0.3	3.6±0.5	3.1±0.4	3.1±0.3		
Water intake (mL/24h)	3.5±0.5	4.1±0.4	3.2±0.3	3.9±0.8		
n	7	12	7	5		

Data from mice provided soy oil diets only. Values are mean±SE. D, disease; S, sex.

Table 4.3. Dietary oil and sex effects in *Pkd2*$^{WS25/-}$ (diseased) mice

	Soy Oil		Flax Oil		Fish Oil		
	Male	Female	Male	Female	Male	Female	P<0.05
Body weight (g)	27.1±1.73	19.3±1.03	27.9±.93	20.8±.60	27.6±1.3	20.6±1.42	S
Kidney							
weight (g)	0.37±0.04^{b}	0.27±0.03^{b}	0.53±0.09^{a}	0.34±0.03^{b}	0.41±0.06^{ab}	0.57±0.10^{a}	I
weight / body weight (g/100g)	1.37±0.11^{b}	1.40±0.12^{b}	1.89±0.33^{b}	1.63±0.15^{b}	1.44±0.14^{b}	2.75±0.37^{a}	I
water content (%)	75.4±1.8^{b}	74.8±1.1^{b}	78.4±2.2^{ab}	75.9±2.3^{b}	75.5±.6^{b}	83.0±1.1^{a}	I
cyst area / section (pixels x10^{3})	468±94	419±99	774±292	566±131	599±145	998±230	
cyst area / kidney area (%)	16.6±5.5	20.9±8.3	23.0±5.9	24.4±5.5	22.6±5.1	32.3±7.2	
Liver							
weight (g)	0.98±0.15	0.77±0.06	1.18±0.06	0.89±0.02	1.18±0.06	0.93±0.06	S
weight / body weight (g/100g)	4.25±0.09	3.95±0.13	4.22±0.10	4.30±0.14	4.29±0.09	4.50±0.15	
water content (%)	66.7±0.6	67.2±2.0	65.9±1.4	66.8±1.1	65.6±0.4	66.1±1.0	
cyst area / liver area (%)	0.24±0.20	0.07±0.03	0.07±0.03	0.21±0.12	0.09±0.03	0.69±0.33	
Feed intake (g/24h)	3.1±0.4	3.1±0.3	2.6±0.1	3.2±0.4	2.3±0.2	4.2±0.6	
Water intake (mL/24h)	3.2±0.3	3.9±0.8	5.2±0.5	4.8±0.4	3.7±0.5	3.4±0.3	
n	7	5	5	7	8	4	

Values are mean±SE. With significant interaction effects, differing lower case superscript letters indicate significant simple effect differences between values. I, interaction; S, sex.

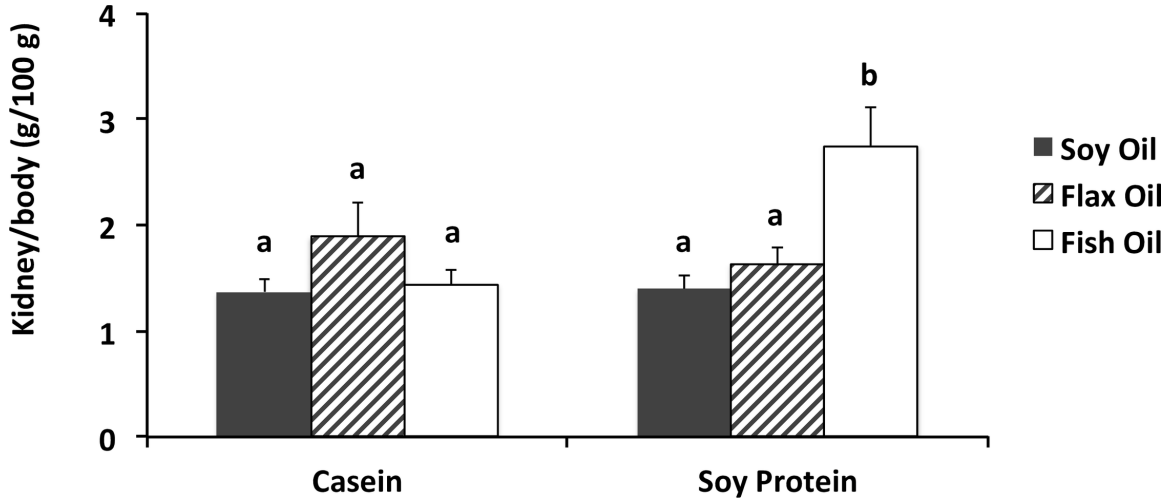

Figure 4.3. **Dietary oil and sex effects on kidney size in *Pkd2*$^{WS25/-}$ (diseased) mice.** There was a diet x sex interaction and differing lower case superscript letters indicate significant simple effect differences between values. Data from Table 2.

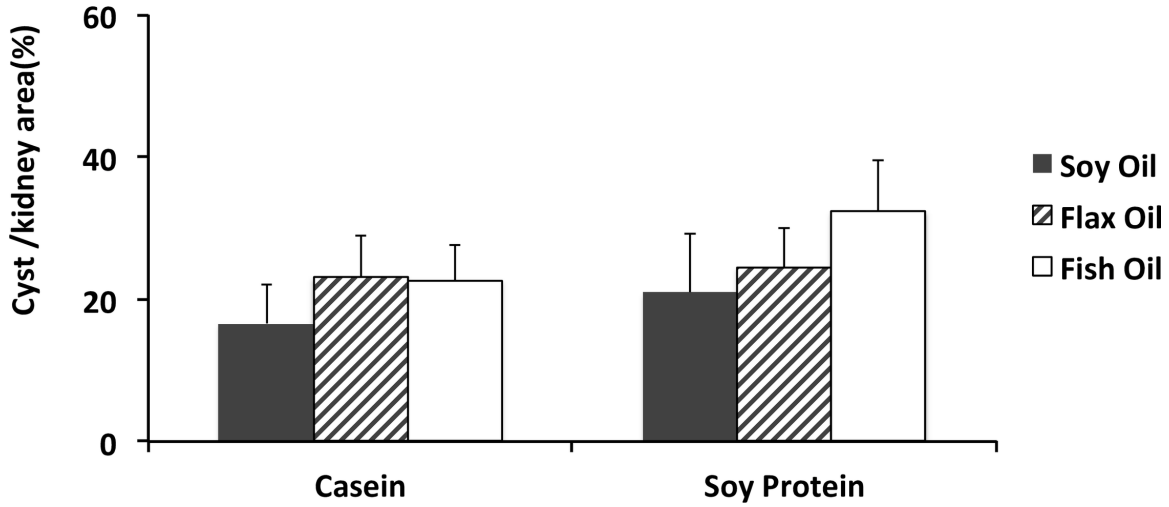

Figure 4.4 **Dietary oil and sex effects on renal cyst area in *Pkd2*$^{WS25/-}$ (diseased) mice.** There were no significant diet or sex effects. Data from Table 2.

4.4.2 PCK rats

PCK rats developed both kidney (Figure 4.1 E-H) and liver cysts (Figure 4.1 E-H) by 16 weeks of age. Renal cysts developed to a greater extent than hepatic cysts, with the cyst and lumen areas being ~ 4 times higher in kidneys compared to livers (Table 4.4). In contrast to *Pkd2*$^{WS25/-}$ mice, fibrosis was detected in the livers but very little fibrosis was observed in the kidneys of PCK rats. The presence of cysts was reflected in higher kidney and liver weights and higher lumen and cyst area, as shown in normal and PCK rats given the control soy oil diet (Table 4.4). PCK rats also had higher urine protein levels and water intake, and lower feed intake and body weights than the normal rats, while serum creatinine, creatinine clearance, urine volume and urine pH were not different (Table 4.4).

PCK rats given fish and flax oil compared to soy oil had larger kidneys with higher water content, kidney cyst and lumen area and creatinine clearance. Providing fish oil compared to both flax and soy oil resulted in larger livers. Rats given flax oil had higher urine pH than those given soy, but not fish oil. There were no dietary oil effects on body weight, feed intake, water intake, serum creatinine, urine protein, urine volume, blood pressure, liver cyst area or liver fibrosis (Table 4.5 and Figure 4.5 and 4.6).

In contrast to *Pkd2*$^{WS25/-}$ mouse models of ADPKD, providing soy protein compared to the casein protein found in the standard AIN93G diet resulted in higher relative kidney weights and lower liver weights in PCK rats. Dietary soy protein also resulted in higher water intake, creatinine clearance, urine pH and urine volume, and in lower urinary protein. Soy protein did not alter body weight, renal water content, renal cyst and lumen area, liver water content, liver cyst area, liver fibrosis, feed intake, serum creatinine or blood pressure (Table 4.5).

Table 4.4. Disease effects in male PCK rats

	Normal	**Diseased**
Body weight (g)	778±15	594±07*
Kidney		
weight (g)	3.8±0.1	4.4±0.1*
weight / body weight (g/100g)	0.49±0.01	0.75±0.01*
water content (%)	76.0±0.3	79.1±0.1*
lumen or cyst area area / section (pixels x 10^3)	16±5	40±5*
lumen or cyst area / kidney area (%)	6.6±2.3	17.6±2.4*
Serum creatinine (mg/dL)	0.46±0.03	0.45±0.03
Creatinine clearance (mL/min)	26.8±4.4	20.8±2.7
Liver		
weight (g)	26.7±0.9	28.3±0.9
weight / body weight (g/100g)	3.44±0.11	4.78±0.16*
water content (%)	61.8±1.0	69.2±0.7*
fibrosis area / liver area (%)	0.2±0.0	3.5±0.3*
lumen or cyst area / liver area (%)	1.4±0.4	4.7±0.2*
Feed intake (g/24h)	30.0±1.9	24.1±0.6*
Water intake (mL/24h)	17.2±0.9	21.0±0.6*
Urine	10.3±0.9	10.1±0.9
pH	5.9±0.0	6.2±0.3
protein/creatinine (mg/mg)	0.9±0.2	14.7±3.8*
volume (mL/24h)	9.0±0.7	9.2±0.7
Mean arterial pressure (mmHg)	117.5±6.9	116.9±5.2
n	8	8

Data from mice provided soy oil diets only. Values are mean±SE. *Significantly different from normal, $P<0.05$.

Table 4.5. Dietary oil and protein effects in diseased PCK rats

	Soy Oil		Flax Oil		Fish Oil		
	Casein	Soy protein	Casein	Soy protein	Casein	Soy protein	P<0.05
Body weight (g)	594±7	592±11	604±7	569±9	576±7	579±13	
Kidney							
weight (g)	4.4±0.1^{B}	4.9±0.2	5.7±0.4^{A}	5.2±0.2	5.4±0.3^{A}	5.9±0.3	O
weight / body weight (g/100g)	0.75±0.01^{C}	0.83±0.03	0.87±0.02^{B}	0.92±0.02	0.94±0.04^{A}	0.99±0.04	O P
water content (%)	79.1±0.1^{B}	79.8±0.4	81.5±0.8^{A}	81.0±0.4	80.4±0.5^{A}	81.0±0.4	O
lumen or cyst area/section (pixels x10^{3})	40±5^{B}	48±5	67±8^{A}	55±10	60±9^{A}	62±6	O
lumen or cyst area/kidney area (%)	17.6±2.4	18.4±1.9	22.3±2.5	21.2±3.6	21.7±3.0	20.7±1.6	
Liver							
weight (g)	28.3±0.9^{B}	27.0±1.1	32.1±1.4^{B}	24.7±0.7	35.5±2.9^{A}	27.8±1.4	O P
weight / body weight (g/100g)	4.78±0.16^{B}	4.56±0.14	5.32±0.26^{B}	4.34±0.09	6.16±0.49^{A}	4.81±0.26	O P
water content (%)	69.2±0.7^{ab}	71.4±0.6^{a}	70.1±0.4^{ab}	68.8±0.4^{b}	70.5±0.8^{ab}	69.7±0.5^{ab}	I
fibrosis area / liver area (%)	3.5±0.3	4.0±0.5	4.6±0.7	3.3±0.3	4.7±0.9	3.7±0.4	
lumen or cyst area / liver area (%)	4.7±0.2	3.9±0.5	6.1±0.8	5.4±1.1	6.0±0.9	4.9±0.8	

Feed intake (g/24h)	24.1±0.6	23.3±1.4	25.3±0.5	24±1	25.6±1.0	25.3±1.1		
Water intake (mL/24h)	21.0±0.6	27.4±1.8	22.8±1.1	26.5±1.09	23.0±1.8	29.3±1.6		P
Serum creatinine (mg/dL)	0.45±0.03^{A}	0.44±0.02	0.49±0.04^{A}	0.41±0.01	0.40±0.03^{B}	0.39±0.04	O§	
Creatinine clearance (mL/min)	20.8±2.7^{B}	23.4±1.7	25.5±2.8^{A}	31.5±4.1	25.1±4.0^{A}	37.1±4.1	O	P
Urine								
pH	6.2±0.3^{B}	7.7±0.4	7.5±0.4^{A}	8.3±0.3	7.3±0.6^{AB}	7.9±0.4	O	P
protein / creatinine (mg/mg)	14.7±3.8	10.1±1.5	10.9±1.6	9.0±1.3	14.2±1.8	9.6±2.1		P
volume (mL/24h)	10.1±0.9	14.9±1.8	12.8±0.8	14.7±0.6	10.0±1.8	14.8±1.4		P
Mean arterial pressure (mmHg)	116.9±5.2	121.4±4.2	129.0±5.0	120.9±4.1	114.6±3.1	117.6±2.8		
n	8	8	8	8	6	8		

Values are mean±SE. With significant dietary oil effects, differing upper case superscript letters in casein columns indicate significant overall (casein and soy protein) differences between groups given different dietary oils. With significant interaction effects, differing lower case superscript letters indicate significant simple effect differences between values. I, interaction; O, oil; P, protein.
P=0.057.

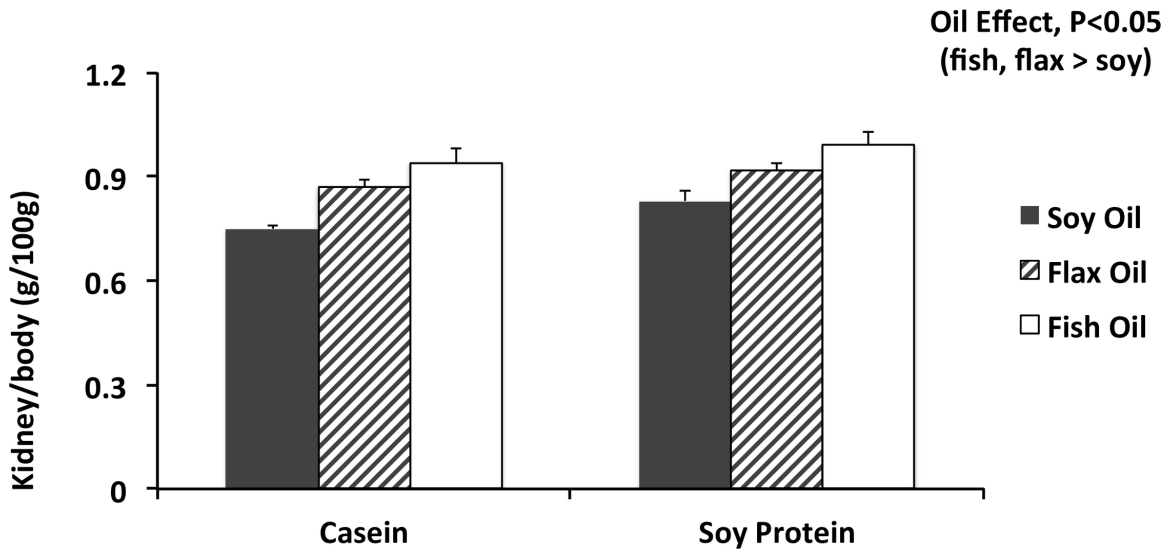

Figure 4.5. Dietary oil and protein effects on kidney size in diseased PCK rats. Significant diet effects are shown on figure. Data from Table 4.

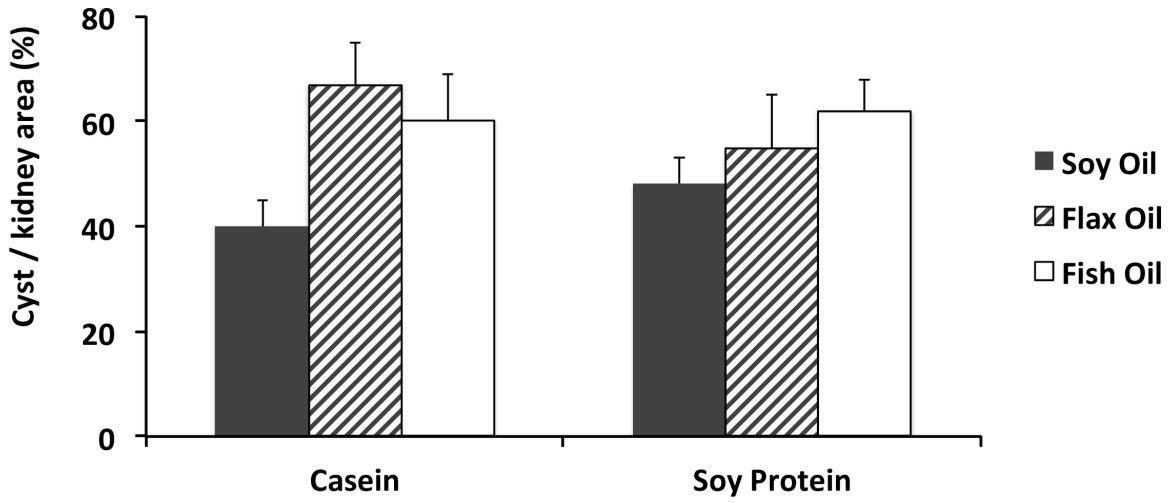

Figure 4.6. Dietary oil and protein effects on renal cyst area in diseased PCK rats. There were no significant diet or sex effects. Data from Table 4.

4.5 Discussion

Overall, dietary interventions with oils enriched in omega-3 fatty acids provided early in the development of PKD displayed no benefits and possible negative effects on disease in both orthologous models of PKD studied. This lack of benefit in male PCK rats and in *Pkd2*$^{WS25/-}$ mice is similar to the findings in female PCK rats(16). In non-orthologous models, fish oil has conflicting effects, with generally protective effects observed in the Han:SPRD-*Cy* rat(10, 510), while in the *pcy* mouse beneficial, detrimental and no effects have been observed (9, 517, 543). With respect to liver effects, fish oil was recently shown to have no effects on liver cysts in female PCK rats, but complications due to cyst obstruction of the bile duct and hepatic vein were evident when these rats were given the fish oil diet (547). Dietary flax oil also displayed no beneficial effects on disease in either orthologous PKD model, which contrasts with the beneficial effects observed in both the Han:SPRD-*Cy* rat and in *pcy* mice (8, 9, 509). Although there were some effects of the oils containing n-3 fatty acids that were detrimental, these were small and not consistent in both models; as well, there were some potentially small positive effects, thus providing insufficient evidence to conclude that these dietary oils were harmful. Overall, these findings do not support dietary advice to increase dietary oils containing n-3 fatty acids for early treatment of PKD, and are consistent with a short-term study in human PKD that failed to demonstrate a beneficial effect of fish oil supplementation (17)

With respect to dietary protein source, there were no differences observed in the *Pkd2*$^{WS25/-}$ mouse. In the PCK rats, although kidney and liver histology were not affected, water intake, creatinine clearance and urine pH were higher, and proteinuria was lower in soy protein fed rats. Similarly, dietary soy protein exhibited no benefits in female PCK rats provided soy protein diets at similar ages (16) Increased water intake in the PCK rat is associated with

reduction in kidney disease progression (548), and increased urine pH via citrate administration is associated with protection from disease in the Han:SPRD-*Cy* rat (549), possibly indicating that there may be benefits of this dietary intervention that had not yet been manifested, but require further study.

While these studies provide no supporting evidence for dietary advice in the early stages of PKD to increase soy protein or oils enriched in omega-3 fatty acids, it is important to determine whether interventions in later stages of disease would benefit from these treatments. Significant benefits of these identical dietary interventions were observed in non-orthologous models, in which disease progression is not only more rapid, but these animals also were terminated at a later stage of disease (7-10, 508). Studies in the Han:SPRD-*Cy* rat when these dietary soy protein, flax or fish oil interventions were initiated after the disease had progressed to the equivalent of approximately stage 3 CKD, dietary interventions also were not as effective, indicating that the intervention may have been too late in the disease process (505). This suggests that there may be a window of opportunity during progressive cyst expansion in which dietary interventions may be more effective.

The amount of soy protein or omega-3 enriched oil also may influence the dietary effect, as the amounts used in the current diets are higher than what would be achievable in analogous human diets. However, the diets used herein were identical in this regard to the studies showing considerable slowing of disease progression in the non-orthologous Han:SRPD-*Cy* rat and *pcy* mouse models (9, 508).

These results also may suggest that dietary interventions may be more effective in models of nephronophthisis (NPHP) compared to models of PKD. The *pcy* mouse is a model of NPHP3 (527), while the Anks6 protein mutated in the Han:SPRD-*Cy* rat appears to form

complexes with NPHP proteins as well (550). Nevertheless, these studies emphasize the need for studies in true models of PKD, and for replicating the results in multiple models, as no one model completely mimics the human form of the disease (526, 551). Investigation of possible sex effects also warrants further investigation.

4.6 Conclusions

These studies show that dietary interventions with soy protein or omega-3 fatty acid enriched oils are not effective when administered during early PKD development in human orthologous models. However, evidence from non-orthologous models indicates that these dietary interventions may possibly be more effective during the progressive growth phase of cyst development.

4.7 Transition to next chapter

The first objective of the current research was to examine if dietary intervention with flax oil, fish oil and soy protein attenuates disease in orthologous models. Both PCK rats (ARPKD model) and Pkd2 mice (ADPKD2 model) used here displayed disease, but the dietary intervention did not result in improvements. Fish oil consumption showed minor detrimental effects.

In the next chapter, objective 1 was examined, in 2 more studies using an orthologous model of ADPKD1 (Pkd1 mice).

Chapter 5

5. Effect of early intervention with dietary oil and protein in the Pkd 1 (Mx1Cre^{+}Pkd1$^{flox/flox}$) mouse model of human ADPKD1

5.1 Introduction

In chapter 4 we have seen the effect of dietary n-3 PUFA containing oils and soy protein on disease in a model of ARPKD (PCK rats) and a model of ADPKD2 with mutations in the *Pkd2* gene (*Pkd2*$^{ws25/-}$ mice). The prevalence of ADPKD is 1:400 to 1:1000, affecting approximately 12.5 million people worldwide (35, 552) and approximately 80-85% of ADPKD is caused by mutations in PKD1, the gene for polycystin 1 (30). Polycystin 1 forms a protein complex, which is localized in primary cilia and the cell-cell matrix of renal cell types (553, 554) and plays an important role in cyst expansion by regulating cAMP levels and modulating cell signaling pathways (554-556). A model with mutations in PKD1 gene, the Mx1Cre^{+}Pkd1$^{flox/flox}$ mouse model, has only been available recently (557, 558). There have been no studies to our knowledge that examined the effect of dietary intervention in this model. The Mx1Cre^{+}Pkd1$^{flox/flox}$ (Pkd1) mouse is a conditional knockout model of ADPKD1. Somatic inactivation of the *Pkd1* gene using polyinosinic polycytidylic acid (pI:pC) injections early in life is reported to develop renal cysts similar to human ADPKD (558). Nonetheless, we decided to examine the effects of dietary interventions on this orthologous model since recent study in an orthologous model of ARPKD was in conflict with results from non-orthologous models (16). Further, since it is not known whether differences exist in disease progression and in the effect of diet, between genders in this model, we used both male and female Pkd1 mice. Therefore, to test whether the benefits of

these dietary interventions in non-orthologous PKD models also occurs in both males and females in ADPKD1 orthologous models, the effects of soy protein compared to casein and flax or fish oil compared to soy oil in Pkd1 mice were examined.

5.2 Materials and Methods

Two independent dietary studies using Pkd1 mice were carried out. All animal procedures were approved by the University of Manitoba Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care.

5.2.1 Study 1 – Mx1Cre^{+}Pkd1$^{flox/flox}$ mouse (5-week induction)

Mx1Cre^{+}*Pkd1*$^{flox/flox}$ (Pkd1) breeders were obtained from Dr. Jing Zhou at Brigham and Women's Hospital and Harvard Medical School (Boston. MA, U.S.A.) (558). Pkd1 mice were either administered injections i.p. once each day with saline for 5 consecutive days beginning at 5 weeks of age to serve as the normal controls, or were treated following the same procedures except they were injected with 250 μ g of polyinosinic polycytidylic acid (pI:pC) to induce somatic inactivation of *Pkd1*. All mice were given diets based on the American Institute of Nutrition (AIN) 93G standard diet for laboratory rodents (541), which has casein as the standard protein source and soy oil as the standard oil. The mice injected with saline were provided only the control AIN93G diet containing casein and soy oil. The diets given to the pI:pC mice were the AIN93G diet or an experimental diet which contained either an equivalent amount of soy protein that replaced the casein, or either flax oil or fish oil that replaced 80% of the soy oil, as shown in Chapter 5 supplementary table 1. Thus mice injected with pI:pC were

provided with diets containing either casein or soy protein as protein source and either soy oil, flax oil or fish oil as the oil source, resulting in a 3-way (sex, protein, oil) design. All oils and diet ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA). The oils contained 0.02% tBHQ to prevent oxidation and diet ingredients were stored at 4˚C. Diet was freshly prepared twice per month and stored in sealed containers at -20˚C until feeding. Routine examination of texture, odor, and color indicated that the oils were not oxidized. Mice were housed singly in a temperature- and humidity-controlled environment with a 12 hour day/night cycle and were given free access to water and diet. All mice were provided these diets for 16 weeks, from 6 to 22 weeks of age.

Mice were weighed bi-weekly and feed and water disappearance and urine volume were measured during week 14 by placing a subset of the mice in metabolic cages for a 36 hour adaptation period followed by a 24 hour data collection period. At termination, body, kidney and liver weights were recorded before placing the left kidney and a portion of the liver in 10% formalin for 24h, followed by transfer to PBS at 4˚C until further processing. The right kidney and another portion of the liver were snap frozen in liquid nitrogen, and lyophilized to determine kidney water content.

Histological procedures were performed similar to PCK rats and Pkd 2 mice in Chapter 5. Formalin fixed kidneys and livers were embedded in paraffin, sectioned at 5 μm and tissue sections were stained with Masson's trichrome to measure cyst area as previously described (9, 510). For cyst area, a Nikon D600 FX DSLR camera equipped with a 60mm F2.8 Macro lens (Nikon Corporation, Mississauga, Canada) was used to capture images of backlit whole kidney sections. Macro rings were used between the camera body and lens to achieve 2.5X magnification. This allowed clear identification of cyst area from complete coverage of the

kidney or liver section in each picture. Quantitative analysis of cyst area of the whole kidney section and of the liver portion section was performed using Image Pro software (Media Cybernetics, Silverspring, MO).

Study 2 – Mx1Cre^{+}Pkd1$^{flox/flox}$ mouse (1-week induction)

The second study used the same mice and treatments as study 1 except for the differences outlined as follows. Pkd1 mice were injected i.p. either with saline or with pI:pC for 5 days beginning at 1 week of age. Dietary treatments were the same as in study 1, except that the protein source was soy protein in all diets, since there was no protein effect in study 1. Test diets were provided for 6 weeks, from 3 to 9 weeks of age. Feed and water disappearance and urine output were determined during week 4 of feeding. All other procedures were as outlined for study 1.

5.2.3 Statistical Analyses

To determine effects of disease and sex, only those provided soy oil (control oil) were compared. In study 1, a 3-way ANOVA (sex x disease x protein) revealed that there were no protein effects on disease, so mice given soy protein and casein were combined for analyses by 2-way (disease x sex) ANOVA. In study 2, mice were only fed soy protein, so disease and sex effects also were tested by 2-way (disease x sex) ANOVA. Dietary effects were tested in diseased animals only, and 3-way ANOVA (sex x oil x protein) conducted for study 1 again revealed that protein had no effect on any parameters. Therefore, mice given soy protein and casein were combined and a 2-way ANOVA (oil x sex) was performed for the diseased mice in study 1. For study 2, a 2-way ANOVA (oil x sex) also was used, as only one protein source was used for all

mice. All ANOVA were performed using the GLM procedure of SAS (SAS, version 9.2, Cary, NC) followed by Duncan's Multiple Range test to delineate significant oil or interaction effects. Normality of the data was assessed using the Shapiro–Wilk's test, and non-normal data normalized by log transforming where possible. If normality was not achieved, data were analyzed using the Kruskal–Wallis test. Statistical significance for main and interaction effects was set at $P < 0.05$. All data are presented as mean±SE.

5.3 Results

5.3.1 Study 1 – Pkd1 mice (5-week induction)

In these Pkd1 mice, initial analyses by 3-way (protein x oil x sex) ANOVA revealed that there were no dietary protein effects on any of the parameters measured. The protein groups therefore were combined to examine the effects of disease, sex and oil. Disease and sex effects were first examined by comparing normal and diseased mice provided the soy oil (control) diets only. Low numbers of small cysts were evident in both kidneys and livers of the diseased mice to a similar extent (Figures 5.1 & 5.2 and Table 5.1), but neither the kidneys nor the livers displayed significant levels of fibrosis. Compared to the normal mice the diseased mice also had lower body weights and higher kidney and liver water content. With respect to sex differences, males had higher body and kidney weights, and female mice had higher kidney water content.

Analyses of dietary oil effects in diseased mice revealed that those given fish oil compared to the soy oil control diet had higher kidney and liver masses, but not kidney or liver cysts, and kidney water content was lower in fish oil fed mice. Flax compared to soy oil fed mice also had lower kidney water content and female mice given flax compared to soy oil had lower liver cyst areas, but no other kidney or liver disease parameters were different between these two

groups. In mice given flax compared to fish oil, kidney weights and total kidney cyst area per section were lower, but liver indicators were not different between these two groups. There were no differences between any of the dietary groups in feed disappearance, water disappearance or urine output measured during the 14th week of feeding or in body weights at the end of the 16 week feeding period (Table 5.2).

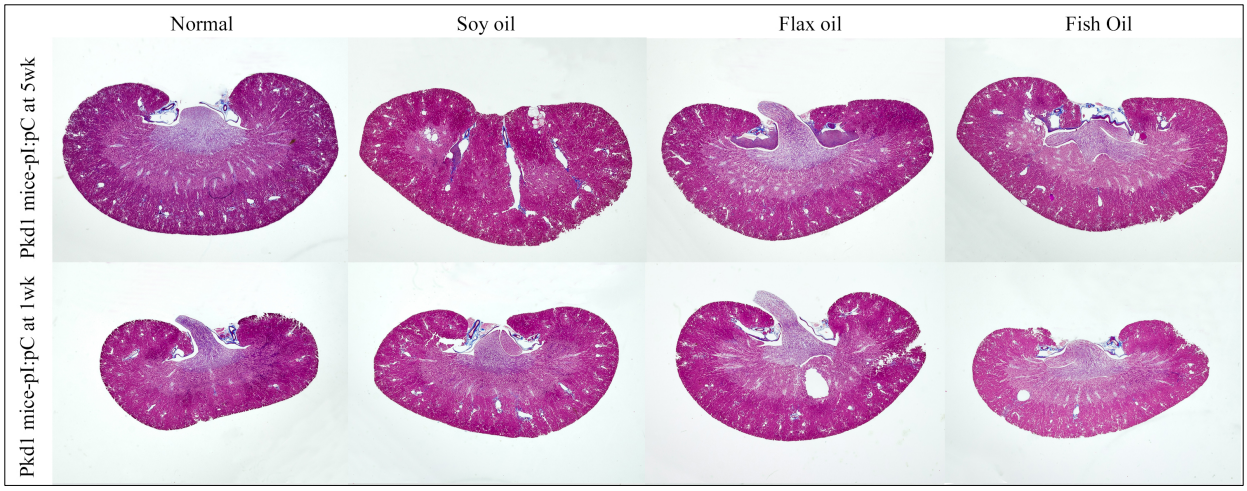

Figure 5.1: **Kidney sections** from Pkd1 mice induced at 5 week (top) and 1 week of age (bottom). Normal is non induced

Figure 5.2: **Liver sections** from Pkd1 mice induced at 5 week (top) and 1 week of age (bottom). Normal is non-induced

Table 5.1. Disease and sex effects in Pkd1 mice administered pI:pC at 5 weeks

	Normal		Diseased			
	Male	Female	Male	Female	P<0.05	
Body weight (g)	30.2±2.2	25.1±1.2	27.8±0.9	22.1±0.6	D	S
Kidney						
weight (g)	0.28±0.02	0.24±0.01	0.28±0.01	0.23±0.01		S
weight / body weight (g/100g)	0.94±0.04	0.97±0.06	1.02±0.04	1.02±0.04		
water content (%)	74.6±0.1	75.1±0.3	74.9±0.2	75.9±0.2	D	S
cyst area / section (pixels x10^3)	-	-	90±9	65±7		
cyst area / kidney area (%)	-	-	4.9±0.5	4.4±0.5		
Liver						
weight (g)	1.12±0.06	1.07±0.07	1.16±0.08	0.95±0.04		
weight / body weight (g/100g)	3.76±0.2	4.30±0.24	4.18±0.25	4.31±0.20		
water content (%)	69.3±0.4	67.2±0.6	70.9±1.3	69.7±0.8	D	
cyst area / liver area (%)	-	-	4.7±0.6	7.2±1.6		
Feed disappearance (g/24h)	3.7±0.4	4.3±0.3	3.6±0.4	4.0±0.3		
Water disappearance (mL/24h)	4.7±1.1	6.7±0.7	6.1±1.2	7.4±0.6		
Urine volume (mL/24h)	2.0±0.6	2.9±0.5	1.5±0.4	3.1±0.6		
n[†]	7	5	12	12		

Data from mice provided soy oil diets only. Values are mean±SE.
D, disease; S, sex.
[†]For feed disappearance, water disappearance and urine volume n=4 for normal and n=8 for diseased groups.

Table 5.2. Dietary oil and sex effects in diseased Pkd1 mice administered pI:pC at 5 weeks

	Soy oil		Flax oil		Fish oil			
	Male	Female	Male	Female	Male	Female	P<0.05	
Body weight (g)	27.8±0.9	22.1±0.6	28.9±1.2	23.2±0.9	27.2±0.9	25.3±0.7		S
Kidney								
weight (g)	0.28±0.01^{B}	0.23±0.01	0.29±0.01^{B}	0.23±0.01	0.32±0.01^{A}	0.30±0.01	O	S
weight / body weight (g/100g)	1.02±0.04^{B}	1.02±0.04	1.02±0.03^{B}	1.01±0.04	1.19±0.04^{A}	1.20±0.02	O	
water content (%)	74.9±0.2^{A}	75.9±0.2	74.4±0.2^{B}	75.0±0.2	74.3±0.2^{B}	75.0±0.2	O	S
cyst area / section (pixels x10^{3})	90±9.0^{AB}	65±7.0	74±6.0^{B}	53±6.0	81±8.0^{A}	94±14	O	
cyst area / kidney area (%)	4.9±0.5	4.4±0.5	4.3±0.4	3.5±0.4	4.5±0.4	5.3±0.7		
Liver								
weight (g)	1.16±0.07^{B}	0.95±0.04	1.27±0.09^{AB}	0.95±0.06	1.25±0.08^{A}	1.12±0.05	O	S
weight / body weight (g/100g)	4.18±0.25	4.31±0.20	4.42±0.26	4.08±0.17	4.59±0.25	4.75±0.19		
water content (%)	69.8±0.8	70.5±0.7	69.7±0.5	68.8±0.5	69.7±0.4	69.1±0.3		
cyst area / liver area (%)	4.7±0.6^{abc}	7.2±1.6^{a}	6.0±0.7^{ab}	3.2±0.3^{c}	3.5±0.4^{bc}	4.7±0.8^{abc}		I
Feed disappearance (g/24h)	3.6±0.4	4.0±0.3	3.4±0.2	3.6±0.3	3.6±0.2	4.2±0.5		
Water disappearance (mL/24h)	6.1±1.2	7.4±0.6	5.0±0.5	6.2±0.7	4.9±0.4	8.8±1.0		S
Urine volume (mL/24h)	1.5±0.4	3.1±0.6	1.4±0.2	2.7±0.6	1.4±0.2	3.1±0.7		S
n†	12	12	12	12	12	12		

Values are mean±SE. With significant dietary oil effects, differing upper case superscript letters in male columns indicate significant overall (male and female) differences between groups given different dietary oils. With significant interaction effects, differing lower case superscript letters indicate significant simple effect differences between values. I, interaction; O, oil; S, sex
†For feed disappearance, water disappearance and urine volume, n = 8.

Study 2 – Pkd1 mice (1-week induction)

To examine whether earlier disease onset along with earlier dietary intervention would alter the dietary effects in this model, mice were induced at 1 week of age and the dietary intervention was carried out from 3 to 9 weeks of age. At the end of the feeding period, kidney and liver cyst areas in the diseased mice were similar in these younger mice compared to the older mice in study 1. However, with the Pkd1 mutation being induced while kidneys are still developing, disease progression was much more variable, particularly in the kidney. Some mice had kidneys with few or no cysts while others displayed large renal cysts (Figures 5.1 & 5.2). In contrast to study 1, no kidney parameters were significantly different in diseased compared to normal mice. However, the diseased mice had larger livers and higher water content, whereas only liver water content was different in study 1 (Table 5.3). The dietary oil effects were minimal, with higher kidney weights in mice given fish oil compared to soy and flax oil, and higher liver weights in fish compared to flax oil mice. There were no differences between flax and soy oil groups. A subset analysis of renal cyst area in only the mice that had cysts also found no dietary effects, confirming a lack of dietary effect on disease progression (Table 5.4).

Table 5.3. Disease and sex effects in Pkd1 mice administered pI:pC at 1 week

	Normal		**Diseased**		P<0.05
	Male	Female	Male	Female	P<0.05
Body weight (g)	21.7±0.9	18.8±0.4	21.85±0.5	19.8±1.0	S
Kidney					
weight (g)	0.24±0.01	0.22±0.01	0.24±0.01	0.24±0.01	
weight / body weight (g/100g)	1.09±0.05	1.17±0.04	1.11±0.05	1.19±0.07	
water content (%)	73.8±0.48	75.0±0.46	74.3±0.8	75.5±0.5	
cyst area / section (pixels x10^3)	-	-	57±14	89±30	
cyst area / kidney area (%)	-	-	3.8±0.9	6.5±2.0	
Liver					
weight (g)	0.88±0.04	0.77±0.03	0.93±0.04	1.01±0.08	D
weight / body weight (g/100g)	4.06±0.18	4.12±0.24	4.25±0.16	5.09±0.26	D
water content (%)	67.35±0.34	67.33±0.29	68.83±0.77	68.37±0.96	D
cyst area/liver area (%)	-	-	4.3±0.5	5.6±0.9	
Feed disappearance (g/24h)	4.1±0.1	4.3±0.7	4.3±0.6	4.4±0.2	
Water disappearance (mL/24h)	6.7±1.4	8.5±2.2	6.6±1.3	6.7±1.0	
Urine volume (mL/24h)	1.2±0.4	3.1	1.6±0.6	2.0±0.5	
n†	7	8	6	4	

Data from mice provided soy oil diets only. Values are mean±SE.

†For feed disappearance, water disappearance and urine volume, n=3, except for urine volume for normal males (n=2) and normal females (n=1).

D, disease; S, sex.

Table 5.4. Dietary oil and sex effects in diseased Pkd1 mice administered pI:pC at 1 week

	Soy oil		Flax oil		Fish oil		
	Male	Female	Male	Female	Male	Female	P<0.05
Body weight (g)	21.9±0.5	19.8±1.0	22.74±0.7	19.1±0.9	23.2±1.1	21.5±0.7	S
Kidney							
weight (g)	0.24±0.01[B]	0.24±0.01	0.26±0.01[B]	0.20±0.02	0.28±0.01[A]	0.27±0.02	O
weight / body weight (g/100g)	1.11±0.05	1.20±0.07	1.13±0.05	1.06±0.1	1.21±0.04	1.23±0.08	
water content (%)	74.3±0.8	75.5±0.5	73.7±0.5	76.3±1.9	74.3±0.7	74.3±0.3	
cyst area / section (pixels x10^3)	57±14	89±30	108±22	35±9	92±24	106±29	
cyst area / kidney area (%)	3.8±0.9	6.5±2.0	7.4±1.4	2.9±0.6	4.3±0.9	8.1±2.2	I[§]
Liver							
weight (g)	0.93±0.04[B]	1.01±0.08	0.95±0.05[B]	0.87±0.13	1.07±0.07[A]	1.10±0.02	O
weight / body weight (g/100g)	4.25±.158	5.09±.261	4.17±.148	4.50±.471	4.62±.251	5.11±.110	S
water content (%)	68.8±0.8	68.4±1.0	68.3±0.4	69.6±0.6	68.0±0.4	68.9±0.5	
cyst area / liver area (%)	4.3±0.5	5.6±0.9	4.5±0.5	3.9±1.5	4.3±0.5	4.2±0.7	
Feed disappearance (g/24h)	11.1±0.5	12.0±0.7	11.3±0.5	10.6±1.0	12.1±0.4	12.4±0.8	
Water disappearance (mL/24h)	6.6±1.3	6.7±1.0	4.9±0.8	7.4±1.5	6.2±0.4	5.55±0.4	
Urine volume (mL/24h)	1.6±0.6	2.0±0.5	1.8±0.5	2.4±0.2	3.2±0.5	3.1±1.0	
n[†]	6	4	7	3	7	4	

Values are mean±SE. With significant dietary oil effects, differing upper case superscript letters in male columns indicate significant overall (male and female) differences between groups given different dietary oils. With significant interaction effects, differing lower case superscript letters indicate significant simple effect differences between values.

§The interaction effect was significant, but no differences between groups were detected with the post hoc (Duncan's) test.

†For feed disappearance, water disappearance and urine volume, n = 3.

I, interaction; O, oil; S, sex

5.6 Discussion

5.6.1 Disease progression in Pkd1 mice

Takakura et al studied the progression of disease in Pkd1 mice and reported massive renal cyst formation 6 weeks after the mice were induced at 1 week of age and moderate cyst formation in 6-9 weeks after induction at 5 weeks of age (558). In our study we used these two time points, but disease progression was not as aggressive in our animals. Although there was higher kidney weight and water content in diseased animals in the 5 week induced mice, generally, there was no difference between normal and diseased in terms of most parameters measured. Time of induction did not have much of an effect on the disease. However, small and variable number and size of cysts were observed in both the studies showing that disease is initiated. The reason for this inconsistency is not clear, and will need to be further examined. With respect to sex effects, there were no differences, possibly due to the fact that the disease was in very early stages.

5.6.2 Effect of dietary intervention

Generally, both dietary soy protein and oils containing n-3 PUFA had minimal effects on disease in this early stage of PKD. 5 week induction study showed no effect of dietary soy protein. This is in contrast with several studies on non-orthologous models that demonstrated consistent benefits with soy protein treatment (4, 5, 7, 10, 506, 559). However, the results from Pkd1 mice are consistent with a recent study on female PCK rats, an orthologous model that showed no benefit of soy protein (16). Our results from male PCK rats and male and female Pkd2 mice (in chapter 5) also showed a similar lack of benefit with soy protein feeding. It appears that the benefits of soy protein are only available in non-orthologous models where disease is further advanced.

Dietary fish oil had some effects, but those were not consistent among parameters. Kidney and liver weights were higher with fish oil feeding indicating that fish oil might be detrimental in disease. However, renal and hepatic cyst areas were not affected by fish oil treatment and there was a reduction in kidney water content. Similar lack of benefit has been previously reported in female PCK rats (16). Again, in non-orthologous models, fish oil has conflicting effects, with largely beneficial outcomes observed in the Han:SPRD-*Cy* rat (10, 510) and beneficial, detrimental and no effects in *pcy* mice (9, 508, 517). A study in humans also failed to show benefits of fish oil supplementation (17). Our results from PCK rats and Pkd2 mice (chapter 5) also support the observation that there is not enough evidence for a recommendation of fish oil consumption in PKD.

Dietary flax oil has consistently shown benefits in non-orthologous models, in both the Han:SPRD-*Cy* rat and in *pcy* mice (8, 9, 509). In our studies, flax oil had lower kidney water content and female mice given flax compared to soy oil had lower liver cyst areas in 5 week induced mice, but no other parameters were affected. Thus our results are not consistent with results from non-orthologous models, but are consistent with results from PCK rats and Pkd2 mice (chapter 5) where no benefit of flax oil was demonstrated. However, it is to be noted that while fish oil showed minor detrimental effects, flax oil did not.

These studies on Pkd1 mice, along with the results from PCK rats and Pkd2 mice provide no evidence to increase the consumption of soy protein or oils containing n-3 PUFA in early PKD. It remains to be elucidated whether benefits will be manifested in later stages of disease, as the non-orthologous models where benefits were observed typically had more advanced disease (7-10, 508).

5.7 Transition to next chapter

The first objective of my research was to examine if beneficial effects of dietary soy protein and n-3 PUFA containing oils are replicated in orthologous models. Chapter 4 described the effects in PCK rats and Pkd2 mice and Chapter 5 examined Pkd1 mice. In all the three orthologous models we studied, there was no clear benefit of these dietary interventions. This lack of benefit was also consistent with a short term human study using fish oil and another recent study on PCK rats using soy protein and fish oil. This was also the first time when flax oil intervention was conducted in an orthologous model, and we did not detect benefits. All the animals in our study were in relatively earlier stages of disease. Therefore, it is concluded that there are no beneficial effects of dietary soy protein, flax oil and fish oil in orthologous models in early disease progression. Whether benefits are yielded in later stages of disease need to be addressed in further research. Our research also calls for reconsidering the dietary advice given to PKD patients, as the basis of current recommendations are results from non-orthologous models.

In the next chapter, the second objective would be addressed which is to determine if there are disease specific alterations in the renal oxylipin profile. Renal oxylipin profiles of normal and diseased animals will be compared to determine any consistent alteration associated with the disease progression. Also, differences in the pattern of oxylipin alteration between ADPKD and ARPKD models will be discussed. To address Objective 4, male and female animals in both normal and diseased groups will be compared to investigate sex effects on renal oxylipin profile.

Chapter 6

6. Distinct Bioactive Lipids Alterations in Diverse Models of Cystic Kidney Diseases

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6.1 Abstract

Cystic kidney diseases are characterized by multiple renal cysts and are the leading cause of inherited renal disease. Oxylipins are bioactive lipids derived from fatty acids formed via cyclooxygenase, lipoxygenase and cytochrome P450 activity. Oxylipins are altered in nephronophthisis, a type of cystic kidney disease. To further investigate and to determine whether other cystic renal diseases share these abnormalities, a targeted lipidomic analysis of renal oxylipins was performed in orthologous models of autosomal dominant polycystic kidney disease 1 (Mx1Cre^{+}*Pkd1*$^{flox/flox}$ mouse) and 2 (*Pkd2*$^{ws25/-}$ mouse) and autosomal recessive polycystic kidney disease (PCK rat). Of over 100 oxylipins screened, 50-55 were detected in each model. Kidney cyclooxygenase oxylipins were consistently higher in all diseased kidneys, even in very early stage disease. On the other hand, cytochrome P450 epoxygenase derived oxylipins were lower only in autosomal recessive polycystic kidney disease. Sex effects on renal oxylipin alterations were observed but they did not always coincide with sex effects on disease. For oxylipins with sex effects, arachidonic acid derived oxylipins formed via cyclooxygenases and lipoxygenases were higher in females, while oxylipins from other fatty acids and via cytochrome P450 enzymes were higher in males. The consistent and unique patterns of oxylipin alterations in the different models indicates the importance of these bioactive lipids in cystic renal diseases, suggesting that pharmacological agents (e.g. cyclooxygenase inhibitors) may be useful in treating this disorder, for which effective treatment remains elusive.

6.2 Introduction

Cystic kidney diseases are a group of inherited renal disorders characterized by proliferation of fluid filled renal cysts in the kidneys and other organs. In humans, the most common forms are ADPKD and ARPKD (553, 560). The prevalence of ADPKD is 1:400 to 1:1000, affecting approximately 12.5 million people worldwide (552, 561). Approximately 85% of ADPKD are caused by mutations in the *PKD1* gene, which codes for polycystin 1 (PC1) protein, and the remaining 15% of cases are caused by mutations in the *PKD2* gene, which codes for polycystin 2 (PC2) protein (35, 562, 563). ARPKD is much less common but is a more severe form of the disease occurring 1 in 20,000-40,000 live births (47, 564). ARPKD presents primarily in infancy and childhood, with 30-50% of patients dying shortly after birth (47, 529, 565). ARPKD results from a defect in the polycystic kidney and hepatic disease-1 (*PKHD1*) gene, which codes for polyductin/fibrocystin (566).

Previous studies with non-orthologous models of PKD have demonstrated alterations in oxylipins in diseased kidneys (10, 18, 567). Oxylipins are bioactive lipid metabolites of PUFA, which are formed by the action of three classes of enzymes: COX, LOX and CYP (8, 78, 568). Oxylipin formation is initiated when PUFA are released from tissue phospholipids by phospholipase A_2, and are subsequently converted into PG and Tx via the COX pathway, hydroxy fatty acids and their metabolites (i.e. Lt and Lx) via the LOX pathway, and epoxy and hydroxy fatty acids via the CYP pathway (8, 77, 81, 569).

Oxylipins are critical for normal renal physiology and function, but also can have detrimental effects in disease. In the *pcy* mouse and Han:SPRD-*Cy* rat models of NPHP, COX enzyme activity and oxylipin levels are higher whereas LOX and CYP oxylipins are lower (8, 10, 18). The mechanism by which the elevated COX oxylipins lead to cyst formation and disease

progression is not clear, but it is known that COX derived oxylipins such as PGE_2 mediate their effects by increasing the levels of cAMP (21, 479, 488, 570-572). Elevated renal cAMP is common in cystic kidneys (557, 573, 574), and several signaling pathways altered in cystic kidney diseases regulate and are regulated in part by cAMP (575-579). The COX oxylipin alterations in *pcy* mouse kidneys occur at a very early age, indicating that these bioactive lipids play a critical role in this disorder.

Whether oxylipins are altered in other types of renal cyst diseases is not clear, however. Human ADPKD displays alterations in some serum oxylipins (580), but renal changes are unknown. The current study therefore undertook a targeted lipidomic analysis of renal oxylipins in diverse models of cystic kidney disease. These analyses reveal that COX oxylipins are consistently elevated across disease models, while CYP epoxygenase oxylipin changes appear to be unique to ARPKD. Novel sex effects on renal oxylipins also were observed.

6.3 Methods

6.3.1 Animal models

Orthologous models of ADPKD1, ADPKD2 and ARPKD were examined in four independent studies. In the first study, Mx1Cre^{+}*Pkd1*$^{flox/flox}$ (Pkd1) mice (ADPKD1 model) (557), were obtained from breeders provided by Dr. Jing Zhou at Brigham and Women's Hospital and Harvard Medical School (Boston. MA, U.S.A). Male and female mice were injected i.p. with 250 μg of polyinosinic polycytidylic acid (pI:pC) to induce somatic inactivation of *Pkd1*or were injected with saline to serve as controls, for five consecutive days beginning at 5

weeks of age. At 23 weeks of age kidneys were harvested. These mice will be referred to as Pkd1 (5wk) mice.

The second study used the same mice as study 1 except that gene inactivation was at 1 week of age and mice were terminated at 9 weeks of age. These mice will be referred to as Pkd1 (1wk) mice.

For the third study, *Pkd2*$^{ws25/ws25}$ and *Pkd2*$^{+/-}$breeders (ADPKD2 model) (544), were obtained from Dr. Stefan Somlo at Yale University (New Haven, CT, U.S.A.). These genotypes were crossed to produce male and female mice with diseased (*Pkd2*$^{ws25/-}$) or normal (*Pkd2*$^{ws25/+}$) phenotypes that were terminated at 16 weeks of age. These mice are referred to as Pkd2 mice. These mice are the control diet mice used in a study published previously on dietary effects on disease (581).

For the fourth study, PCK rats (ARPKD model) (582), were purchased from a commercial breeder (Charles River, QC, Canada). Normal and PCK male (only) rats were terminated at 16 weeks of age. These rats are the control diet rats used in a study published previously on dietary effects on disease (581).

Animals in all studies were housed in temperature and humidity controlled environments with a 12 hour day/night cycle, and were given free access to water and standard semi-purified or chow diets (For fatty acid composition of diets, please see casein+soy oil group in table 7.1 in the next chapter). At termination, body and kidney weights were measured and the right kidney was snap frozen in liquid nitrogen, and stored at -80˚C until oxylipin analysis. The left kidney was fixed by placing it in 10% formalin for 24h, followed by transfer to PBS at 4˚C until further processing. Formalin fixed kidneys were embedded in paraffin, sectioned at 5 μm and tissue sections were stained with Masson's trichrome as previously described (9). All animal procedures

were approved by the Institutional Animal Care Committees and adhered to the guidelines of the Canadian Council on Animal Care.

6.3.2 Oxylipin analysis

Lyophilized whole kidney tissues were homogenized in ice cold Tyrode's salt solution (pH 7.6) in a 1:28 weight/volume ratio. After homogenization Triton X-100 was added to achieve a final concentration of 0.01%. Deuterated internal standards (10ng of each, Cayman Chemical, MI, USA) and 6.5 μL of antioxidant cocktail [0.2 g/L BHT, 0.2 g/L EDTA, 2 g/L triphenylphosphine, and 2 g/L indomethacin in MeOH:EtOH:H_2O (2:1:1,by vol)] were added to 200 μL tissue homogenates used for analysis. Samples were adjusted to pH<3 and solid phase extraction was with Strata-X SPE columns (Phenomenex, CA, USA) preconditioned with methanol and pH 3 water. Samples were loaded onto the columns, rinsed with 10% methanol, and eluted with methanol. Evaporated samples were then re-suspended in solvent for analysis by HPLC-MS/MS (API 4000, AB Sciex, Canada) as described (583), based on methods developed by Deems et al (584), A list of all oxylipins screened, detector response factors and internal standards are listed in Appendix F. Detection and quantification limits were set at 3 and 5 levels above background, respectively. Quantification of oxylipins was determined using the stable isotope dilution method (585), and amounts expressed as pg/mg of dry tissue.

6.3.3 Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS, version 9.4, Cary, NC, USA). Disease and sex effects in mouse studies were tested by 2-way (disease x sex) ANOVA. Male rats only were used in the PCK rat study, so disease effects were tested using t-tests.

Normality of data was tested using Shapiro-Wilk's Statistic (W > 0.05 for normally distributed data). If the data did not follow a normal distribution even if transformed, a nonparametric test was used (Kruskal-Wallis). Post hoc analysis was done by Duncan's multiple range tests for simple effect comparisons when interactions were present or when the Kruskal-Wallis test indicated the presence of differences. All data were presented as mean ±SEM. Significance was set at $p < 0.05$ for main, interaction, and simple effects.

6.4 Results

Cystic kidney disease was present in all models studied, although the extent of disease varied widely between models (Figure 6.1, Table 6.1). Small cysts were present in both models of ADPKD1 [Pkd1 (5wk) or Pkd1 (1wk) mice], but kidney size was not affected in this early stage of PKD, and kidney water was only slightly (<1%) higher in Pkd1 (5wk) mice with disease. Larger cysts were observed in Pkd2 mice and PCK rats, and this was reflected in 20-30% larger kidneys and 3-4% higher water content in diseased kidneys.

Over 100 oxylipins were analyzed for each study and between 50 and 55 oxylipins were detected in normal and diseased PKD kidneys. When oxylipins were examined as totals from the different biosynthetic pathways (Figure 6.2), COX oxylipins were consistently higher in diseased kidneys, being 30%, 21%, 40% and 32% higher in Pkd1 (5wk), Pkd1 (1wk), Pkd2 and PCK models, respectively. In contrast, total CYP epoxygenase derived oxylipins were only lower in PCK rats (58%), whereas total LOX and CYP oxylipins were not affected by disease.
The patterns observed in total COX oxylipins reflected the differences in individual oxylipins (Tables 6.2-6.5). In diseased kidneys, individual COX oxylipins that were significantly higher

were PGE_2 (27% higher) and 6-keto-$PGF_{1\alpha}$ (129% in females only) in Pkd1 (5wk) mice, PGD_2 (20%), PGE_2 (25%) and 6-keto-$PGF_{1\alpha}$ (43% in females only) in Pkd1 (1wk) mice, PGD_2 (260%), $PGF_{2\alpha}$ (49%) and 6-keto-$PGF_{1\alpha}$ (67%) in Pkd2 mice, and PGE_2 (38%) and 6-keto-$PGF_{1\alpha}$

Table 6.1 Disease parameters in orthologous models of PKD

	Normal		Diseased			
	Male	Female	Male	Female	*P>0.05*	
Pkd1 (5wk)						
Body weight (g)	30.2±2.2	25.1±1.2	27.8±0.9	22.1±0.6	D	S
Kidney						
weight (g)	0.28±0.02	0.24±0.01	0.28±0.01	0.23±0.01		S
weight / body weight (g/100g)	0.94±0.04	0.97±0.06	1.02±0.04	1.02±0.04		
water content (%)	74.6±0.1	75.1±0.3	74.9±0.2	75.9±0.2	D	S
Pkd1 (1wk)						
Body weight (g)	21.7±0.9	18.8±0.4	21.85±0.5	19.8±1.0		S
Kidney						
weight (g)	0.24±0.01	0.22±0.01	0.24±0.01	0.24±0.01		
weight / body weight (g/100g)	1.1±0.05	1.2±0.04	1.1±0.05	1.2±0.07		
water content (%)	73.8±0.48	75.0±0.46	74.3±0.8	75.5±0.5		
Pkd2						
Body weight (g)	26.2±2.2	19.5±0.6	27.1±1.7	19.3±1.0		S
Kidney						
weight (g)	0.28±0.02	0.21±0.01	0.37±0.04	0.27±0.03	D	S
weight / body weight (g/100g)	1.1±0.04	1.1±0.04	1.4±0.01	1.4±0.12	D	
water content (%)	72.2±0.6	71.5±1.0	75.4±1.8	74.8±1.1	D	
PCK						
Body weight (g)	778±15		594±07		D	
Kidney						
weight (g)	3.8±0.10		4.4±0.10		D	
weight / body weight (g/100g)	0.49±0.01		0.75±0.01		D	
water content (%)	76.0±0.3		79.1±0.10		D	

Values are mean±SE. ǂmale rats only were used in the PCK study. Values with differing lower case superscript letters indicate simple effect differences between values. D, disease; S, sex; I, interactions. Data compiled from tables in chapter 4.

Figure 6.1 Kidney sections from normal and diseased kidneys from: a. Pkd1 (5wk), b. Pkd1(1wk), c. Pkd2, and d. PCK models

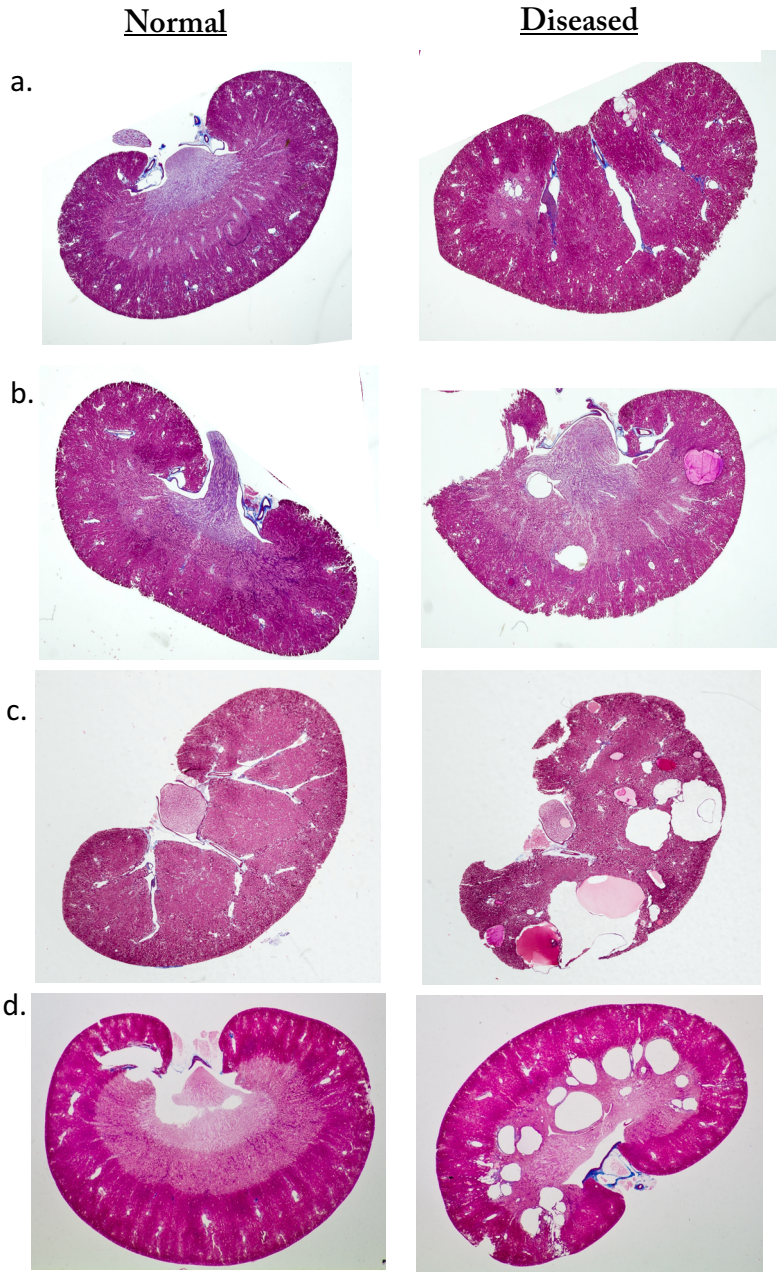
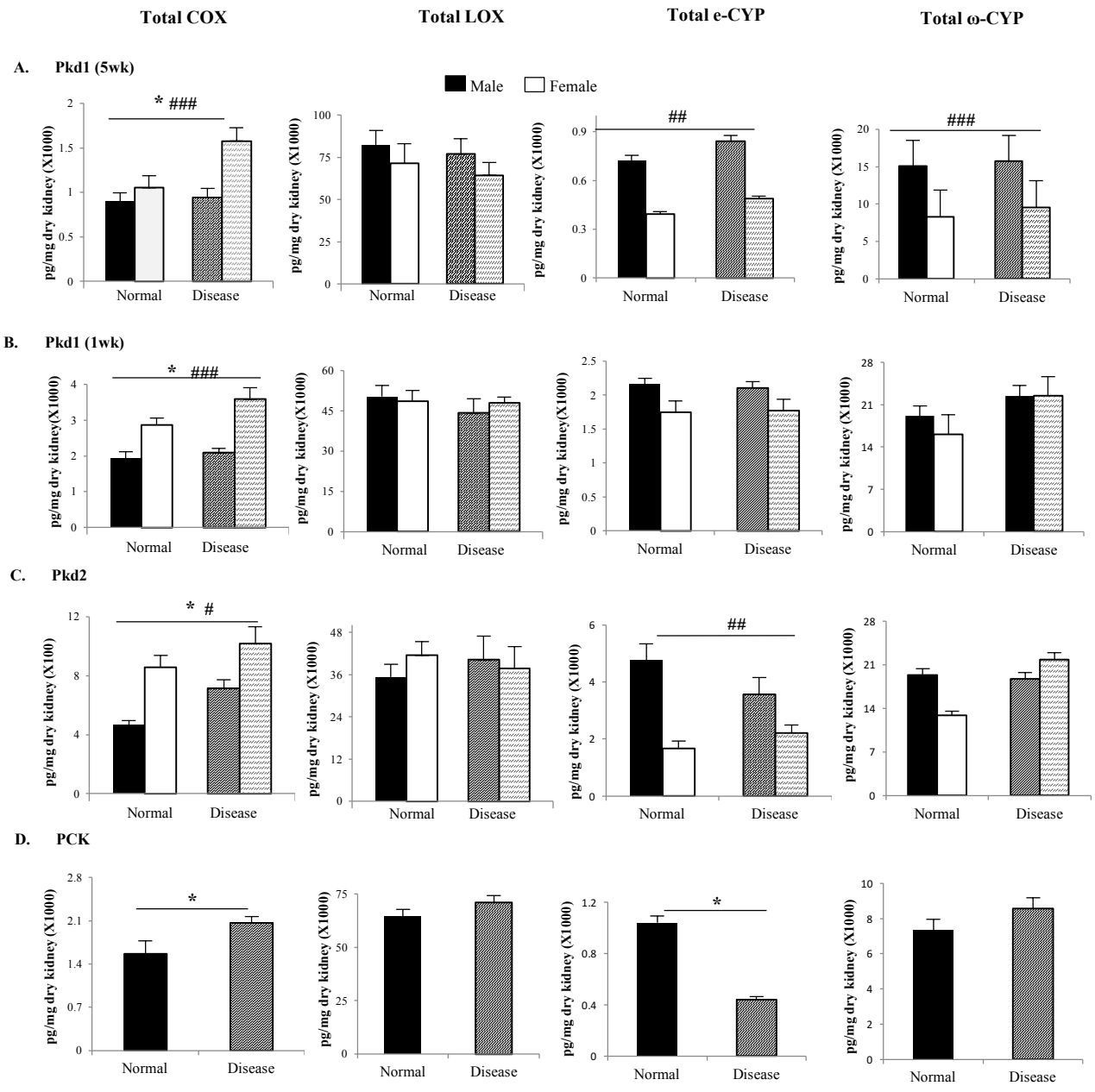


Figure 6.2 Effect of disease and sex on total kidney COX, LOX and CYP derived oxylipins in orthologous models of cystic kidney disease.



denotes disease effect; #denotes sex effect (,# $p<0.05$; **,## $p<0.01$; ***,### $p<0.001$). Values with differing lower case superscript letters indicate simple effect differences between values.

Table 6.2: Renal oxylipins in normal and diseased Pkd1 (5wk) (Mx1Cre^{+}*Pkd1*$^{flox/flox}$) mice.

Oxylipin (pg/mg dry tissue)	PUFA	Normal		Diseased		P<0.05	
		Male (n=7)	Female (n=5)	Male (n=6)	Female(n=6)		
COX Pathway							
PGD_2	AA	175±31.4	192±18.6	166±33.3	203±31.5		
PGE_2	AA	202±20.1	346±34.7	279±24.8	419±17.6	D	S
$PGF_{2\alpha}$	AA	206±37.1	287±38.7	217±25.9	354±29.3		S
6-keto-$PGF_{1\alpha}$	AA	310±71.5^{b}	332±66.1^{b}	302±50.4^{b}	760±122^{a}	I	
LOX Pathway							
9-HODE	LA	5941±379	3754±122	4451±270	4137±559		S
9-oxo-ODE	LA	2815±350	2175±426	1840±195	2531±304		
13-HODE	LA	5857±445	4080±247	5097±692	5256±586		
13-oxo-ODE	LA	2708±349	2690±657	2856±336	2212±438		
9,10,13 TriHOME	LA	3806±511	3069±706	3243±518	2869±330		
9,12,13 TriHOME	LA	1439±156	1110±286	1142±160	946±124		
8-HETrE	DγLA	286±26.6	213±63.7	265±29.4	152±15.5		S
15-HETrE	DγLA	117±21.0	96.4±16.3	88.7±20.6	113±15.5		
5-HETE	AA	1763±146	1623±94	1828±173	1740±197		
5-oxo-ETE	AA	371±34.0	306±16.3	411±50.5	390±53.4		
8-HETE	AA	353±56.8	510±106	445±78.4	424±37.8		
9-HETE	AA	785±164	1068±218	818±162	1177±110		
11-HETE	AA	813±151	1023±85.5	798±172	1299±100		S
12-HETE	AA	905±235	1152±163	1267±284	1657±269		
15-HETE	AA	1348±189	1717±205	1428±206	2008±137		S
15-oxo-ETE	AA	1874±322	1785±415	1556±282	1519±328		
9-HOTrE	ALA	678±119	368±76.1	528±76.9	303±59.0		S
9-oxo-OTrE	ALA	234±61.7	262±143	189±21.3	266±90.6		
13-HOTrE	ALA	43291±7088	40556±11761	39852±8629	28892±4349		
5-HEPE	EPA	405±88.7	413±138	382±59.8	300±106		
8-HEPE	EPA	109±35.7	175±106	79.8±9.40	72.5±26.1		

9-HEPE	EPA	653±188	639±233	667±130	519±178		
11-HEPE	EPA	224±61.5	363±143	237±34.4	214±59.0		
12-HEPE	EPA	524±77.5	485±74.3	761±88.3	509±126		
15-HEPE	EPA	264±39.7	214±48.1	285±52.4	300±50.3		
4-HDoHE	DHA	2147±158	1088±103	2330±184	1342±219		S
7-HDoHE	DHA	222±33.9	157±24.1	179±38.4	145±18.8		
10-HDoHE	DHA	231±60.1	197±34.8	158±23.2	139±27.3		
10S,17S DiHDoHE	DHA	56.0±11.1	28.2±8.20	68.3±6.50	30.9±5.70		S
11-HDoHE	DHA	461±80.7	425±75.5	419±86.6	391±56.1		
13-HDoHE	DHA	177±28.1	127±15.2	185±26.6	141±13.2		
14-HDoHE	DHA	767±123	574±67.5	792±94.6	646±87.5		
16-HDoHE	DHA	776±86.7	526±53.8	846±43.9	565±59.6		S
17-HDoHE	DHA	2528±318	1492±79.0	2431±107	1666±181		S
17-keto-DHA	DHA	2219±603	1167±218	3025±857	1013±141		S
CYP Pathway							
9,10-DiHOME	LA	99.6±6.40	67.2±7.20	94.6±10.9	75.2±8.10		S
12,13-DiHOME	LA	63.4±4.70	42.9±5.50	61.3±7.60	45.8±3.10		S
5,6-DiHETrE	AA	125±15.0	78.7±10.9	126±12.7	96.6±15.6		S
8,9-DiHETrE	AA	19.6±1.80	15.5±1.50	17.3±1.80	17.3±2.10		
11,12-DiHETrE	AA	44.4±6.20	39.1±1.80	41.7±6.30	46.8±5.40		
14,15-DiHETrE	AA	41.7±4.20	51.1±5.70	50.4±6.20	59.0±4.70		
16-HETE	AA	198±24.3	168±19.5	184±17.8	183±13.8		
18-HETE	AA	45.9±5.40	39.1±6.50	42.2±5.50	30.6±2.20		
18-HEPE	EPA	549±20.6	358±50.7	586±40.2	499±39.2	D	S
19,20-DiHDPE	DHA	330±48.7	100±13.3	454±59.7	150±9.70	D	S
20-HDoHE	DHA	14331±1406	7691±669	14926±1548	8832±1018		S

Values are expressed as mean ± SEM. Values with differing lower case superscript letters indicate simple effect differences between values. D, disease; S, sex; I, interactions.

Table 6.3: Renal oxylipins in normal and diseased Pkd1 (1wk) (Mx1Cre^{+}*Pkd1*$^{flox/flox}$) mice.

Oxylipin (pg/mg dry tissue)	PUFA	Normal		Diseased		P<0.05	
		Male (n=6)	Female (n=8)	Male (n=6)	Female (n=4)		
COX Pathway							
PGD_2	AA	477±51.9	798±43.9	518±62.1	1064±78.2	D	S
PGE_2	AA	192±16.7	400±49.3	261±22.7	482±30.9	D	S
$PGF_{2\alpha}$	AA	241±23.4	333±29.9	240±19.5	419±42.9		S
6-keto-$PGF_{1\alpha}$	AA	425±45.9^{c}	680±57.3^{b}	418±26.7^{c}	976±54.2^{a}	I	
TxB_2	AA	31.5±2.95	44.1±7.99	28.7±4.87	58.7±8.02		S
PGE_3	EPA	8.80±1.70	8.68±2.18	8.47±2.88	11.1±1.58		
Δ17-6-keto-$PGF_{1\alpha}$	EPA	1.24±0.13	1.26±0.24	0.94±0.13	1.48±0.18		
LOX Pathway							
9-HODE	LA	3803±254	4090±395	4037±385	4201±256		
9-oxo-ODE	LA	1483±210	2051±158	1769±301	1973±243		
13-HODE	LA	4737±266	5068±684	4520±459	5265±610		
13-oxo-ODE	LA	4622±619	6174±553	4036±531	5939±783		S
9,10,13 TriHOME	LA	2047±235	1729±193	1523±102	1892±222		
9,12,13 TriHOME	LA	671±53.3	589±67.8	526±48.1	611±88.7		
13-HOTrE-γ	γLA	56.2±7.22	42.6±5.75	50.9±6.04	50.0±7.34		
8-HETrE	DγLA	156±18.5	153±13.2	163±8.02	164±13.9		
15-HETrE	DγLA	90.5±6.90	61.1±4.59	89.8±7.16	82.9±10.2		S
5-HETE	AA	2017±304	2225±159	1830±176	2669±244		S
5-oxo-ETE	AA	346±79.4	546±34.5	295±38.6	558±80.0		S
8-HETE	AA	737±101	955±83.6	847±69.0	913±175		
9-HETE	AA	1164±119	1195±124	1213±127	1603±71.8		
11-HETE	AA	542±116	879±65.9	625±45.1	945±196		S
12-HETE	AA	1077±154	877±104	1150±83.9	1332±127	D	

15-HETE	AA	746±80.6	1077±93.6	831±60.0	1427±131	D	S
9-HOTrE	ALA	325±45.2	228±38.6	277±75.1	236±38.8		
9-oxo-OTrE	ALA	210±51.2	201±38.6	138±42.8	256±145		
13-HOTrE	ALA	20187±1827	19023±2562	13979±2715	13385±1153	D	
5-HEPE	EPA	392±62.8	392±41.4	376±36.2	401±32.9		
8-HEPE	EPA	115±28.4	99.4±16.4	76.5±14.2	64.1±10.8		
9-HEPE	EPA	279±55.6	248±28.2	252±20.6	294±35.4		
11-HEPE	EPA	147±21.5	131±16.1	121±14.0	149±25.6		
12-HEPE	EPA	426±48.7	268±26.9	442±49.6	398±25.4	D	S
15-HEPE	EPA	96.5±15.5	78.7±11.3	72.1±13.8	81.6±11.7		
4-HDoHE	DHA	989±98.1	837±69.9	1098±115	1076±129		
7-HDoHE	DHA	230±51.6	149±28.6	203±25.6	190±13.5		
8-HDoHE	DHA	959±110	659±77.6	988±123	896±116		
10-HDoHE	DHA	134±16.7	114±16.2	136±10.3	145±21.1		
10S,17S-DiHDoHE	DHA	26.0±2.40	22.1.0±4.07	29.6±4.29	29.4±6.0		
11-HDoHE	DHA	218±33.4	175±22.7	238±29.2	219±15.9		
13-HDoHE	DHA	81.0±8.35[ab]	66.1±6.56[b]	82.4±5.44[ab]	97.5±4.37[a]	I	
14-HDoHE	DHA	531±7.9	340±38.0	511±45.1	505±40.3		
16-HDoHE	DHA	481±61.1	347±35.9	454±35.3	479±38.6		
17-HDoHE	DHA	1010±120	742±103	969±97.1	1001±70.2		
17-keto-DHA	DHA	490±104	524±67.8	481±43.1	622±68.8		
RvD_2	DHA	97.2±16.2	108±15.5	102±11.8	93.2±17.2		
CYP Pathway							
9,10-DiHOME	LA	102±8.11	79.9±9.74	83.9±10.0	82.0±5.31		
12,13-DiHOME	LA	1708±64.5	1457±167	1655±219	1421±70.0		
5,6-DiHETrE	AA	48.5±5.70	37.1±5.31	61.3±5.28	66.9±10.8	D	
8,9-DiHETrE	AA	14.1±2.22	14.4±1.71	13.4±1.11	18.8±2.56		
11,12-DiHETrE	AA	40.6±4.98	39.9±6.08	34.4±3.33	49.3±4.63		
14,15-DiHETrE	AA	36.4±3.82	36.2±3.97	38.1±2.02	44.7±4.06		
16-HETE	AA	57.0±3.69	59.5±2.47	49.5±5.97	59.8±3.95		
18-HETE	AA	2.87±1.04	2.57±0.58	1.87±0.53	2.24±0.86		

18-HEPE	EPA	1233±137	1218±181	1382±158	1446±122		
19,20-DiHDPE	DHA	207±22.8	85.4±8.06	222±22.6	94.1±10.7		S
20-HDoHE	DHA	17862±1478	14853±2044	21038±1899	20938±1975	D	
Non-enzymatic products							
8-iso-$PGF_{2\alpha}$III	AA	588±48.3	810±81.7	621±50.7	1030±68.3		S

Values are expressed as mean ± SEM. Values with differing lower case superscript letters indicate simple effect differences between values. D, disease; S, sex; I, interactions.

Table 6.4: Renal oxylipins in normal (*Pkd2$^{ws25/+}$*) and diseased (*Pkd2$^{ws25/-}$*) Pkd2 mice.

Oxylipin (pg/mg dry tissue)	PUFA	Normal		Disease		*p<0.05*	
		Male (n=6)	Female (n=4)	Male (n=3)	Female (n=3)		
COX Pathway							
PGD_2	AA	31.8±18.0	75.6±63.6	133±24.1	256±72.6	D	
PGE_2	AA	180±28.1	355±72.3	229±11.0	395±63.4		S
11β-PGE_2	AA	482±68	741±124	632±55	1054±353		S
$PGF_{2\alpha}$	AA	123±8.70	227±42.0	177±12.9	346±38.1	D	S
6-keto-$PGF_{1\alpha}$	AA	283±23.7	654±130	672±188	895±120	D	S
TxB_2	AA	47.9±7.4	88.9±8.1	58.7±15.3	102±12.7		S
LOX Pathway							
9-HODE	LA	4170±533	4129±800	4755±1118	5480±1532		
9-oxo-ODE	LA	1672±189	949±264	918±116	602±31.9		
13-HODE	LA	4995±662	4910±853	5963±1598	5780±1409		
13-oxo-ODE	LA	2243±365	1447±662	3463±870	2432±702		
9,10,13-TriHOME	LA	2209±176	1692±329	2639±595	2936±312	D	
9,12,13-TriHOME	LA	1068±95.8	887±128	1032±79.5	1221±219		
8-HETrE	DγLA	193±31.0	109±24.2	123±53.3	111±6.30		
15-HETrE	DγLA	226±37.7	98±19.0	159±85.4	102±17.2		
5-HETE	AA	1119±179	1448±425	1121±445	2028±158		
5-oxo-ETE	AA	281±68.4	265±96.7	118±8.70	293±21.2		
8-HETE	AA	271±34.7	277±102	355±124	530±125		
9-HETE	AA	637±119	619±213	520±200	772±147		
11-HETE	AA	407±62.6	518±147	296±38.0	516±31.2		
12-HETE	AA	1885±471	556±159	1213±544	1624±619		
15-HETE	AA	987±130	1436±345	674±162	1117±57.7		
15-oxo-ETE	AA	574±44	660±370	624±157	629±128		
9-HOTrE	ALA	177±47.7	173±27.1	106±0.5	288±154		
9-oxo-OTrE	ALA	87.2±34.8	46.6±10.3	69.4±4.7	95.1±23.3		
13-HOTrE	ALA	10638±1944	8283±865	12127±5602	12497±7051		

5-HEPE	EPA	182±35.5	152±5.7	247±92.5	279±67.6		
9-HEPE	EPA	166±39.0	60.2±7.4	145±54.6	81.4±7.0		S
11-HEPE	EPA	603±116	191±28	275±134	308±100		
12-HEPE	EPA	603±116	191±28	275±134	308±100		
4-HDoHE	DHA	1357±214	751±152	750±288	901±116		
7-HDoHE	DHA	156±19.7	81.6±8.80	117±45.1	106±14.3		
8-HDoHE	DHA	649±97.3	374±130	628±221	551±34.2		
10-HDoHE	DHA	108±18.4	70.1±10.8	57.8±4.9	49.2±2.6	D	
11-HDoHE	DHA	344±58.3	186±21.6	137±26.8	128±7.60	D	
13-HDoHE	DHA	150±19.6	89.4±17.2	88.3±22.8	79.6±9.80		
14-HDoHE	DHA	926±160	376±69.8	395±99.2	383±64.0		
16-HDoHE	DHA	726±88.0	423±60.3	647±196	521±37.9		
17-HDoHE	DHA	1591±235	999±75.7	1125±187	1027±91.9		
17-keto-DHA	DHA	2061±454	927±92.6	1077±351	848±143		
CYP Pathway							
12,13-DiHOME	LA	67.3±5.9	37.9±5.5	111±24.9	93.0±34.7	D	
9,10-DiHOME	LA	73.1±6.70	46.8±8.70	77.2±10.7	99.5±34.7		
16-HETE	AA	124±16.9	96.0±22.6	79.4±42.2	69.4±16.1		
18-HETE	AA	99.9±36.1	73.6±48.6	38.7±20.1	18.9±3.70		
19-HETE	AA	412±66.6	391±82.3	521±53.6	551±276		
14,15-DiHETrE	AA	39.2±3.40	30.8±4.60	33.7±5.20	32.6±1.90		
5,6-DiHETrE	AA	91.7±10.4	77.6±12.9	61.5±31.4	84.6±4.70		
11,12-DiHETrE	AA	28.1±2.40	25.7±7.0	28.0±9.70	35.1±0.90		
18-HEPE	EPA	598±104	396±145	715±111	800±301		
19,20-DiHDPE	DHA	649±105	113±14.2	401±96.7	96.7±6.30		S
20-HDoHE	DHA	18142±2121	11959±3212	17406±3124	20371±1616		
Non-enzymatic product							
5-iso PGF2αVI	AA	14.1±3.30	26.3±5.90	24.8±4.70	44.8±4.60	D	S

Values are expressed as mean ± SEM. Values with differing lower case superscript letters indicate simple effect differences between values. D, disease; S, sex.

Table 6.5: Renal oxylipins in normal and diseased PCK rats

Oxylipin (pg/mg dry tissue)	PUFA	Normal (n=8)	Diseased (n=8)	P<0.05
COX Pathway				
PGD_2	AA	248±45	283±34	
PGE_2	AA	180±13	250±12	D
11β-PGE_2	AA	552±131	572±30	
$PGF_{2\alpha}$	AA	355±45	382±29	
6-keto-$PGF_{1\alpha}$	AA	239±19	366±24	D
TxB_2	AA	218±3	289±34	
LOX Pathway				
9-HODE	LA	8825±934	13512±1377	D
9-oxo-ODE	LA	8955±1252	7344±524	
13-HODE	LA	9818±539	15001±1249	D
13-oxo-ODE	LA	5332±763	3827±314	
9,10,13 TriHOME	LA	5198±401	4739±763	
9,12,13 TriHOME	LA	1825±168	1833±318	
13-HOTrE-γ	γLA	280±21	367±63	
8-HETrE	DγLA	389±63	483±23	
15-HETrE	DγLA	218±19	325±23	D
5-HETE	AA	4375±494	4719±352	
5-oxo-ETE	AA	1087±149	613±61	D
8-HETE	AA	1158±194	999±45	
9-HETE	AA	2876±440	3121±188	
11-HETE	AA	3784±508	3884±270	
12-HETE	AA	1595±219	1881±128	
15-HETE	AA	4850±653	6269±395	
15-oxo-ETE	AA	3992±484	2591±217	D
9-HOTrE	ALA	598±57	870±113	
9-oxo-OTrE	ALA	382±59	325±76	
13-HOTrE	ALA	61214±3753	90212±11000	

5-HEPE	EPA	2424±530	1573±190	
9-HEPE	EPA	522±86	416±57	
11-HEPE	EPA	191±32	166±21	
12-HEPE	EPA	154±21	205±15	
15-HEPE	EPA	424±51	823±111	D
4-HDoHE	DHA	948±138	880±73	
7-HDoHE	DHA	230±34	124±16	D
10-HDoHE	DHA	168±26	114±9	
11-HDoHE	DHA	423±57	376±31	
13-HDoHE	DHA	101±17	94±9	
14-HDoHE	DHA	472±81	499±43	
16-HDoHE	DHA	360±65	342±30	
17-HDoHE	DHA	1481±250	1520±122	
CYP Pathway				
9,10-DiHOME	LA	190±22	141±21	
12,13-DiHOME	LA	94.0±11	32.0±4.0	D
5,6-DiHETrE	AA	181±30	51.0±8.0	D
8,9-DiHETrE	AA	103±31	41.0±3.0	D
11,12-DiHETrE	AA	130±15	74.0±10	
14,15-DiHETrE	AA	186±32	56.0±4.0	D
16-HETE	AA	362±20	285±23	D
18-HETE	AA	126±15.0	121±20.0	
18-HEPE	EPA	801±124	1431±148	D
19,20-DiHDPE	DHA	157±55.0	48.0±5.0	D
20-HDoHE	DHA	6083±995	6740±371	
Non-enzymatic products				
5-iso-$PGF_{2\alpha}$ VI	AA	313±30	259±20	
8-iso-$PGF_{2\alpha}$	AA	844±126	1088±61	D

Values are expressed as mean ± SEM. D, disease.

(58%) in PCK rats. Consistent with the lack of change in total LOX oxylipins, there were few and inconsistent changes in individual LOX oxylipins in all the models studied. The lower total CYP epoxygenase oxylipins in disease in PCK rat kidneys also were reflected in the significantly different individual oxylipins, in which 5/7 CYP epoxygenase oxylipins were lower in disease.

With respect to sex effects on renal parameters, the ADPKD models exhibited few differences between male and female mice. Female Pkd1 (5wk) had higher kidney and water weights, but no effect on kidney weight relative to body weight. There were no sex effects on these parameters in Pkd1 (1wk) mice, and only on kidney weight (lower in female) in Pkd2 mice.

Oxylipins levels were affected by sex, with all oxylipins that had a sex effect being higher in males, except for almost all oxylipins derived from AA. In Pkd1 (5wk) mouse kidneys, for example, 6 AA derived oxylipins were affected by sex, and 5 of these were higher in females, while in comparison, all 13 oxylipins derived from other fatty acids that were affected by sex were higher in males. In Pkd1 (1wk) and Pkd2 mice, the analogous numbers were 6/6 and 10/10 AA oxylipins with a sex effect, respectively, being higher in females, and 2/2 and 3/4 non-AA derived oxylipins, respectively, being higher in males. These ADPKD models displayed minor or no effects of sex on disease, and there were few interactions of sex with disease for oxylipins.

Interestingly, the AA oxylipins that were higher in females all were derived via the COX or LOX pathways, or produced non-enzymatically. No AA oxylipins derived via the CYP pathways were higher in females. For the non-AA oxylipins with a sex effect, they were derived from all enzymatic pathways, although few derived from the COX pathway were detected. Hence, total COX oxylipins were higher in females, while total LOX oxylipins had no sex effect, and CYP oxylipins either had no sex effect, or were higher in male kidneys (Figure 6.2).

6.5 Discussion

The present findings demonstrate that COX oxylipins are consistently higher in diseased kidneys in orthologous models of ADPKD1, ADPKD2 and ARPKD, in agreement with previous findings in two non-orthologous models, the Han:SPRD-*Cy* rat (10, 513, 585, 586), and the *pcy* mouse (8, 567). This suggests that increased levels of these bioactive lipids is a common feature of all cystic renal diseases. COX oxylipins were elevated even in early stage disease (i.e. Pkd1 models), which is consistent with previous findings in early stage disease in *pcy* mice (567), suggesting that these alterations are critical during early disease development.

Oxylipins in the kidney play key regulatory roles in normal physiological function, maintaining GFR and salt/water homeostasis, as well as being involved in inflammatory and proliferative processes in response to renal injury (587-589). The precise roles of COX oxylipins in cystic renal diseases are not known, but they are known to be involved in several key pathogenic pathways in the development of these disorders. One such pathway is related to cAMP and subsequent signaling pathways, as renal cAMP is elevated in several models of PKD (573, 574, 590), and cAMP stimulates epithelial cell proliferation and fluid secretion in human kidney cyst cells (487). Lipid extracts containing COX oxylipins from *pcy* mouse renal cyst fluid stimulate secretion, cAMP production and cell proliferation in Mardin-Darby Canine Kidney cells (591). A recent study showed that downregulation of cAMP by inhibiting histone deacetylase 6 reduces cyst growth and proliferation of cyst-lining epithelial cells in a Pkd-conditional mouse (578). COX oxylipins may influence these cAMP mediated events via stimulation of their cognate G protein-coupled receptors and the resulting production of cAMP (488, 571, 575, 577).

This stimulation of cAMP production by COX oxylipins such as PGD_2, E_2 and I_2 also may influence several other altered signaling molecules in cystic kidney diseases, such as arginine vasopressin (AVP) and microRNA-21 (miR-21). AVP is a major regulator of adenylyl cyclase activity and source of cAMP production in the distal nephron (579). AVP receptor antagonists inhibit ERK-dependent cell proliferation and in vitro cyst growth of human ADPKD cells (592), whereas AVP receptor agonists restore the full cystic phenotype in the AVP null PCK rat (593). AVP receptor activity also is dependent on functional COX-2 activity (594). AVP receptor agonists increase renal cAMP levels and aggravate disease development in animal models (65), whereas, AVP receptor antagonists inhibit disease development in animal models (595-598) and in ADPKD patients (599).

cAMP signaling also transactivates renal cell miR-21 (575), which is upregulated in several orthologous mouse models of ADPKD as well as in human ADPKD kidneys (575, 578). Programmed cell death 4 (PDCD4) tumor suppressor gene is the target for miR-21 (600), and inactivation of miR-21 results in increased expression of PDCD4 in cyst epithelial cells (575). PDCD4 is a pro-apoptotic gene and a subset of PDCD4 null (*Pdcd4*$^{-/-}$) mice spontaneously develop kidney cysts (601). In relation to the current findings, in vitro treatment with PGE_2 up-regulates miR-21 expression and down-regulates PDCD4 proteins in colonic adenocarcinoma cells, while selective COX-2 inhibition reverses this (600).

In contrast to increased production of renal COX oxylipins in all models, levels of LOX oxylipins were not affected by disease. This is in contrast with findings in non-orthologous models where lower LOX products were observed (8, 10, 18). In the *pcy* mouse, LOX oxylipin changes were observed later in disease than the changes in COX oxylipins (18), indicating that these changes may be a consequence of disease, but this was not observed in our studies.

Reduced CYP epoxygenase oxylipins were observed in the PCK rat, but not in Pkd1 and Pkd 2 mice suggesting that there are unique differences in oxylipins in ADPKD and ARPKD. The fact that the patterns in all 3 ADPKD models were similar (higher COX oxylipins only) and different from that in PCK rats (higher COX and lower CYP epoxygenase oxylipins) indicates that these two types of cystic renal disease have distinct patterns of oxylipins alterations. Studies with other models of ARPKD are needed to confirm whether the unique oxylipin pattern in the PCK rat (higher COX and CYP epoxygenase oxylipins) also occurs in other models of this cystic kidney disease.

It is possible that the differences in the extent of disease could have contributed to the different oxylipin patterns observed in the models used in this study. However, the fact that the level of disease in Pkd2 mice and PCK rats was similar, but the oxylipin patterns were distinct, suggests that this was not likely a major contributor the different oxylipin patterns. Further evidence to this comes from a previous study in 60 days old *pcy* mice (18), in which LOX alterations were different than in the Pkd2 mice and PCK rats in the current study, even though the disease severity in the 60 day old *pcy* mice was similar to that observed in the Pkd2 mice and PCK rats. Studies in the same models over time are needed to determine the precise role, if any, that disease severity has on the pattern of oxylipin alterations in each type of cystic kidney disease.

In all models which included both sexes, total oxylipins derived via the COX pathway were higher in females, consistent with the higher levels of renal COX oxylipins in females that has been reported previously (531, 533, 534). However, this observation may be due to the fact that the COX oxylipins in the previous and current studies were predominantly derived from AA. Interestingly, AA derived oxylipins with a sex effect that were produced via the LOX

pathways also were higher in females, while oxylipins with a sex effect derived from non-AA fatty acids were higher in males, suggesting that the sex effect may be influenced by the fatty acid substrate for these pathways. In contrast, all CYP oxylipins with a sex effect were higher in males. These sex differences may be due to sex differences in the level of available fatty acid substrate (602-604), enzyme preference for specific fatty acids for oxylipin synthesis or degradation (531, 536, 605), or differences in transporters and excretion of oxylipins from the kidney (537). Some evidence for all of these mechanisms has been reported; however, the evidence is scant and primarily restricted to AA oxylipin data. Further studies are clearly needed to confirm the current findings and to further elucidate potential mechanisms regulating the effect of sex on renal oxylipin formation. Nevertheless, these sex effects on oxylipins do not appear to explain sex differences early in disease progression, as the very small sex effects on disease in the ADPKD models were not consistent with sex effects on oxylipins.

In conclusion, COX oxylipins are consistently elevated across different types and severity of cystic kidney diseases, suggesting that inhibition of these bioactive lipids may help slow disease progression. Effective treatment to prevent or slow cystic kidney diseases remains elusive (29, 504, 606), but the current findings and the fact that COX inhibitors reduce disease progression in the Han:SPRD-*Cy* rat, as well as other models of renal disease (486, 585, 607-609), suggests that use of these inhibitors in very early disease may offer a potential therapeutic approach in these disorders. The other unique patterns of oxylipin alterations involving the CYP epoxygenase pathway in the pediatric form of disease also may provide opportunities for therapeutic interventions in these disorders

6.6 Transition to next chapter

In chapter 6, objectives 2 and 4 were discussed. For the first time, a comprehensive profile of more than 50 oxylipins present in the renal tissue of both normal and diseased, male and female animals were reported in both mice and rats with PKD. With disease, consistent elevation in COX derived oxylipins were observed in all three models studied. Three individual oxylipins, namely PGD_2, PGE_2 and 6-keto-$PGF_{1\alpha}$ were identified to be higher in disease animals. These results are consistent with previous reports on non-orthologous models. A novel finding was that the pattern of oxylipin alteration is distinct between ARPKD and ADPKD models. Another novel insight is the unique effect of sex on AA oxylipins, which was higher in females whereas other oxylipins were either similar or higher in males.

The next chapter will address objective 3, which was to determine the effect of dietary treatments on the renal oxylipin profile. Additionally, chapter 7 will also investigate whether the sex effects observed in chapter 6 are confirmed in diseased animals.

Chapter 7

7. Distinct effects of dietary flax compared to fish oil, soy protein compared to casein, and sex on the renal oxylipin profile in models of polycystic kidney disease

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7.1 Abstract

Oxylipins are bioactive lipids derived from polyunsaturated fatty acids (PUFA) that are important regulators of kidney function and health. Targeted lipidomic analyses of renal oxylipins from four studies of rodent models of renal disease were performed to investigate the differential effects of dietary flax compared to fish oil, soy protein compared to casein, and sex. Across all studies, dietary fish was more effective than flax oil in reducing n-6 PUFA derived oxylipins and elevating eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived oxylipins, whereas dietary flax oil resulted in higher α-linolenic acid (ALA) oxylipins. Dietary soy protein compared to casein uniquely resulted in higher linoleic acid (LA) derived oxylipins. Kidneys from females had higher levels of arachidonic acid (AA) oxylipins, but similar or lower levels of oxylipins from other PUFA. These unique dietary and sex effects on the oxylipin profile may help elucidate their effects on renal physiology and health.

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; COX, cyclooxygenase; CYP, cytochrome P450; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DiHDoHE, dihydroxy-docosahexaenoic acid; DiHDPE, dihydroxy-docosapentaenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; DiHOTrE, dihydroxy-octadecatrienoic acidEPA, eicosapentaenoic acid; EpOME, epoxy-octadecenoic acid; GLA, gamma-linolenic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; LA, linoleic acid; LOX, lipoxygenase; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; oxo-OTrE, oxo-octadecatrienoic acid; PG, prostaglandin; Rv, resolvin; TriHOME, trihydroxy-octadecenoic acid; Tx, thromboxane.

7.2 Introduction

Oxylipins are oxygenated metabolites of polyunsaturated fatty acids (PUFA) formed by mono- or dioxygen-dependent reactions of cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) enzymes (19). Although eicosanoids formed from arachidonic acid (AA) are the most widely studied class of oxylipins, recent advances in lipidomics have revealed a large number of novel oxylipins formed from other PUFA with chain length varying from 18 (octadecanoids) to 22 carbons (docosanoids) (19, 584, 610, 611). This class of bioactive lipids plays significant roles in many key physiological processes in kidney health and disease, including maintaining blood flow, hemodynamics, renin secretion, and glomerular filtration rate (476, 478, 480). Oxylipins also are involved in inflammatory, fibrotic and proliferatory events in diseased kidneys (21, 481).

Dietary oils influence the production of oxylipins in tissues, including the kidney. Increased dietary intake of n-3 PUFA has long been associated with an increase in beneficial n-3 prostaglandins (PG) and a reduction in n-6 PG (612), but the distinct effects of different n-3 PUFA [i.e. α-linolenic acid (ALA) vs. eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)] on health (613-615) may be due to unique effects of their oxylipin metabolites. In kidney, few oxylipins have been studied, particularly in relation to dietary fish oil intake. In rats, a limited number of n-3 PUFA derived renal oxylipins were reported to be elevated and n-6 PUFA oxylipins were reduced with fish oil consumption in two recent studies (10, 616). With flax oil feeding, elevated levels of several oxylipins from ALA and EPA and lower levels of some n-6 oxylipins have been reported in kidneys from obese rats (121). In a mouse model of renal disease, flax oil feeding lowered AA and linoleic acid (LA) oxylipin levels and elevated ALA,

EPA and DHA oxylipins in renal tissues (8). These findings are generally similar to human plasma oxylipin studies with fish (468, 613, 617) and flax oil (471, 514), which have examined a much wider range of oxylipins. However, neither the human plasma studies nor the rodent renal studies have compared the effects of fish to flax oil on the oxylipin profile directly in the same model. Additionally, a detailed profile of oxylipins in the kidney is lacking.

Much less is known about effects of dietary protein on oxylipins. We recently reported that soy protein compared to casein feeding reversed disease associated alterations in a small number of oxylipins produced from n-6 PUFA in rat renal tissues (10). Peng et al also demonstrated a reduction in AA derived levels of thromboxane B_2 (TxB_2), and 6-keto $PGF_{1\alpha}$ in rat renal tissues with soy protein feeding that also was associated with diseased mitigation (513). These results suggest that soy protein could alter renal oxylipins, but it is not clear whether this is an indirect effect due to its effects on disease. On the other hand, soy protein can reduce the activity of Δ6 desaturase, an enzyme that converts LA to longer chain PUFA, and raise the LA levels (521, 522), and thus could potentially alter the formation of oxylipins from these fatty acids.

The effect of sex on oxylipins also is largely unknown, but there are some indications of differences between male and female oxylipin levels in the kidney. Female rats have higher renal levels of AA derived PGE_2, which may be due to lower levels of a PG-specific transporter responsible for PG clearance in rat renal tissues (537). Higher levels of PGE_2 and TxB_2 in the urine of diseased female rats have also been reported (533). Gender differences in enzymes that metabolize AA and AA oxylipins also may be responsible for these differences (531, 532, 535, 536, 605). These limited results indicate that there are sex specific differences in renal oxylipins,

but a comprehensive analysis of differences in the male and female oxylipin profile has not been performed in the kidney, or any other tissue.

We recently reported that there were few effects of dietary fish and flax compared to soy oil, soy protein compared to casein, and sex on disease progression in a rat and a mouse model of polycystic kidney disease (618). We also examined these dietary and sex effects in 2 other studies with another model of this disease, but in these studies the disease progression was insufficient to examine dietary effects on disease. Since the dietary interventions and sex had only minor or no effects on disease in all four of these studies, the kidneys were examined herein to determine the effects of these diet interventions and sex on the comprehensive renal oxylipin profile. Findings across the different studies consistently revealed that fish and flax oil have distinct effects on the renal oxylipin profile, that soy protein increases LA derived oxylipins, and that AA derived oxylipins are uniquely higher in females.

7.3 Materials and methods

7.3.1 Animal models

Kidneys from four studies of rodent polycystic kidney diseases were used for the analyses. The first and second studies used $Mx1Cre^{+}Pkd1^{flox/flox}$ (Pkd1) conditional knockout mice from our in-house colony, originally provided by Jing Zhou (Brigham and Women's Hospital and Harvard Medical School, Boston. MA, U.S.A) (557). To induce disease, male and female Pkd1 mice in the first study were administered i.p. with 250 μg polyinosinic polycytidylic acid (pI:pC) for five consecutive days beginning at 5 weeks of age [hereafter called Pkd1 (5wk) mice], and in the second study were injected at 1 week of age [hereafter called Pkd1 (1wk) mice]. For the third

study, Pkd2$^{ws25/ws25}$ and Pkd2$^{+/-}$breeders were obtained from Dr. Stefan Somlo (Yale University, New Haven, CT, USA) (544) and crossed to produce (Pkd2$^{ws25/-}$) mice with disease (hereafter called Pkd2 mice). In the fourth study, weanling male PCK rats (582) purchased from a commercial breeder (Charles River, QC, Canada) were used.

7.3.2 Diets

Diets were based on the American Institute of Nutrition (AIN) 93G standard diet for laboratory rodents (541) and reported in detail in the previously published study on disease effects in PCK rats and Pkd2 mice (618). All four studies had diets containing either soy oil, flax oil or fish oil with the only difference between these diets being that flax or fish oil replaced 80% of the soy oil in the standard soy oil diet (details in Table 4.1 and Table 7.1). Thus dietary oil effects were examined in all four studies. The studies with Pkd1 (5wk) mice, Pkd2 mice and PCK rats also had diets that replaced the standard protein source (casein) with soy protein, resulting in 6 different diets (Table 4.1). In Pkd2 mice, however, diseased mice could only be identified upon termination and the number of mice in the protein groups was found to be too low in some subgroups to test protein effects on oxylipins, so protein effects were examined only in Pkd1 (5wk) mice and PCK rats. The three mouse studies also included both males and females, allowing the examination of sex effects in these studies. All diet ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA). Oils contained 0.02% tert-butylhydroquinone (added by Dyets Inc) to prevent oxidation and diet ingredients were stored at 4˚C. Diet was freshly prepared twice per month and stored in sealed containers at -20˚C until feeding.

Animals were housed singly in a temperature and humidity controlled environment with

a 12-hour day/night cycle and were given free access to water and diet. The feeding period for each study was 16 weeks (6 to 22 weeks of age), 6 weeks (3 to 9 weeks of age), 13 weeks (3 to 16 weeks of age) and 12 weeks (4 to 16 weeks of age), for the Pkd1 (5wk), Pkd1 (1wk), Pkd2 mice and PCK rats, respectively. At the end of each study, animals were anesthetised with isoflurane. Mice were terminated by decapitation and PCK rats were terminated by cardiac puncture. The right kidney was snap frozen in liquid nitrogen and stored at -80˚C until analysis. All animal procedures were approved by the University of Manitoba Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care.

Table 7.1 Fatty acid composition of the diets

	Casein			Soy protein		
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil
C14:0	0.23	0.24	5.69	0.34	0.43	7.38
C16:0	11.47	7.21	20.13	13.74	10.66	19.17
C16:1t	0	0.02	0.62	0.03	0.02	0.35
C16:1	0.13	0.12	10.42	0.27	0.41	9.4
C17:1	0.05	0.03	1.19	0.06	0.03	1.01
C18:0	3.97	3.48	4.1	4.26	3.71	4
C18:1	20.95	17.77	10.43	19.68	17.7	9.58
C18:1n7c	1.16	0.65	2.64	1.24	0.81	2.47
C18:2	53.28	23.24	14.29	51.39	24.96	17.58
C18:3n6	0	0.02	0.55	0	0.01	0.49
C18:3n3	7.39	46.01	3.14	6.85	39.98	3.47
C20:1	0.19	0.2	0.74	0.18	0.22	0.62
C20:4	0	0.01	0.93	0.01	0.02	0.88
C20:5n3	0	0	11.46	0.03	0	10.12
C22:5n3	0	0	1.92	0.05	0.01	1.82
C22:6n3	0	0.01	8.74	0.44	0.09	8.55

Values are g/100g fatty acid (n=2-3 per diet)

7.3.3 Oxylipin analysis

Lyophilized whole kidney tissues were homogenized in ice cold Tyrode's salt solution (pH 7.6) in a 1:28 weight:volume ratio. After homogenization, Triton X-100 was added to achieve a final concentration of 0.01%. Deuterated internal standards (10 ng each, Cayman Chemical, MI, USA) and 6.5μL antioxidant cocktail [0.2 g/L BHT, 0.2 g/L EDTA, 2 g/L triphenylphosphine, and 2 g/L indomethacin in MeOH:EtOH:H_2O (2:1:1,by vol)] were added to 200 μL aliquots that were used for analysis. Samples were adjusted to pH < 3 and solid phase extraction was with Strata-X SPE columns (Phenomenex, CA, USA) preconditioned with methanol and pH 3 water. Samples were loaded onto the columns, rinsed with 10% methanol, and eluted with methanol. Evaporated samples were then resuspended in solvent for analysis by HPLC-MS/MS (API 4000, AB Sciex, Canada) as described (583) based on methods developed by Deems et al. (584). Details of all oxylipins screened, the deuterated internal standards used and the detector response factors are listed in Appendix F. Detection and quantification limits were set at 3 and 5 levels above the background, respectively. Quantities of oxylipins were determined using the stable isotope dilution method (619) and expressed as pg/mg dry tissue.

7.3.4 Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS, version 9.4, Cary, NC, USA). For Pkd1 (5wk) mice, a 3-way (sex x protein x oil) ANOVA was performed and for the Pkd1 (1wk) mice and Pkd2 mice, a 2 way (sex x oil) ANOVA was used. For the PCK rat study, protein and oil effects were tested by 2-way (protein x oil) ANOVA.

Normality of data was tested using Shapiro-Wilk's Statistic (W > 0.05 for normally

distributed data). Non-normal data was transformed to achieve normality. If the data did not follow a normal distribution even when transformed, a nonparametric test was used (Kruskal-Wallis). Post hoc analysis was done by Tukey-Kramer tests for simple oil effect comparisons when interactions were present or when the Kruskal-Wallis test indicated the presence of treatment differences. All data were presented as mean ± standard error (SE). Significance was set at $p < 0.05$ for main, interaction, and simple effects.

7.4 Results

A total of 158 oxylipins were scanned for in each study (Appendix F), of which 61 were detected at quantifiable levels [49 in Pkd1 (5wk) mice, 54 in Pkd1 (1wk) mice, 50 in Pkd2 mice and 51 in PCK rats].

7.4.1 Effect of dietary lipids on renal n-6 PUFA derived oxylipins

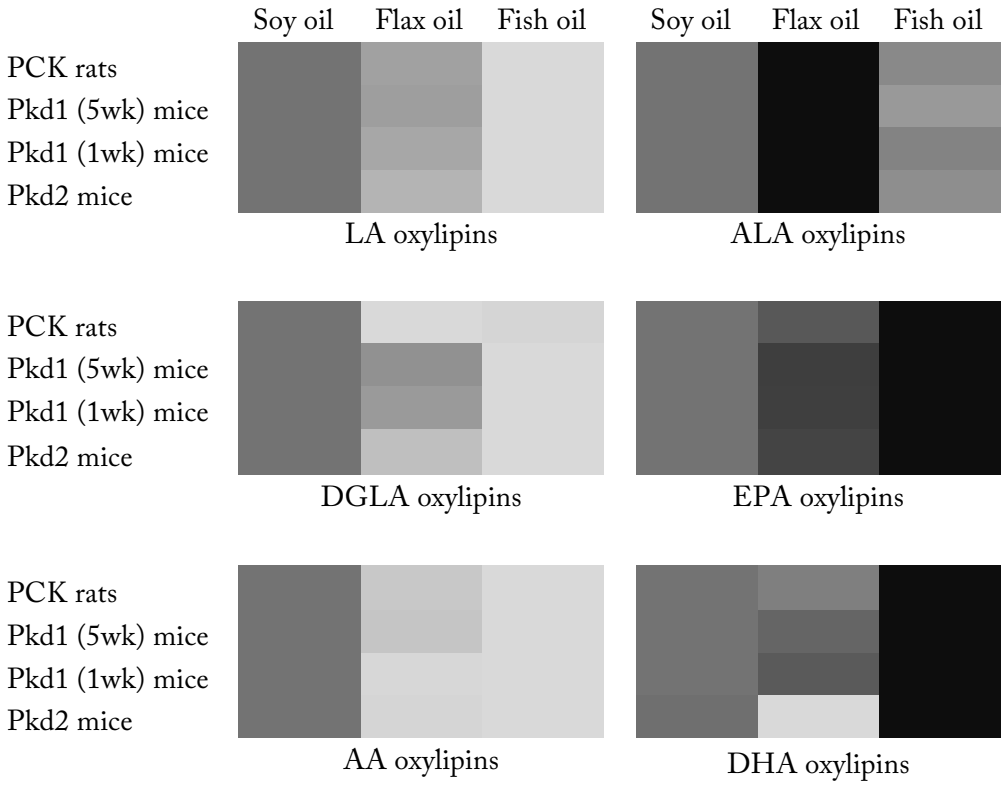
Kidney oxylipins derived from n-6 PUFA were generally lowest in the fish compared to the soy oil groups, with the levels in the flax oil groups being intermediate. Across all four of the studies, total LA oxylipins in all three dietary oil groups were different from each other. Compared to the soy oil groups, total LA oxylipins were 26-42% lower in the flax oil and 59-66% lower in the fish oil groups, while in fish compared to the flax oil groups, they were 40-54% lower (Figure 7.1, Tables 7.2-7.5). Of the 8 individual LA oxylipins quantified in each study, 25-75% were lower in the flax and 100% were lower in the fish compared to the soy oil groups, across all four studies. Fish oil had a greater lowering effect than flax oil in 25-100% of individual LA oxylipins (Tables 7.6-7.9).

Similar to the LA oxylipins, total renal AA oxylipins in flax compared to soy oil groups

had 37-44% lower total AA oxylipins in the three mouse models, and 57% lower levels only in the casein fed PCK rats. As well, individual AA oxylipins were 53-62% lower in the fish compared to soy oil groups across all studies. In contrast to the consistently lower LA oxylipins across all studies, total AA oxylipins were lower (by 20%) only in Pkd1 (5wk) mice in fish compared to flax oil fed animals (Tables 7.2-7.5). With respect to individual oxylipins, 17-19 individual AA oxylipins were quantitated in each of the four studies. 74-100% of these individual oxylipins were lower in flax, and 100% of were lower in fish compared to soy oil fed animals across all four studies, while 6-29% of individual AA oxylipins were lower in fish compared to flax oil fed animals (Tables 7.6-7.9).

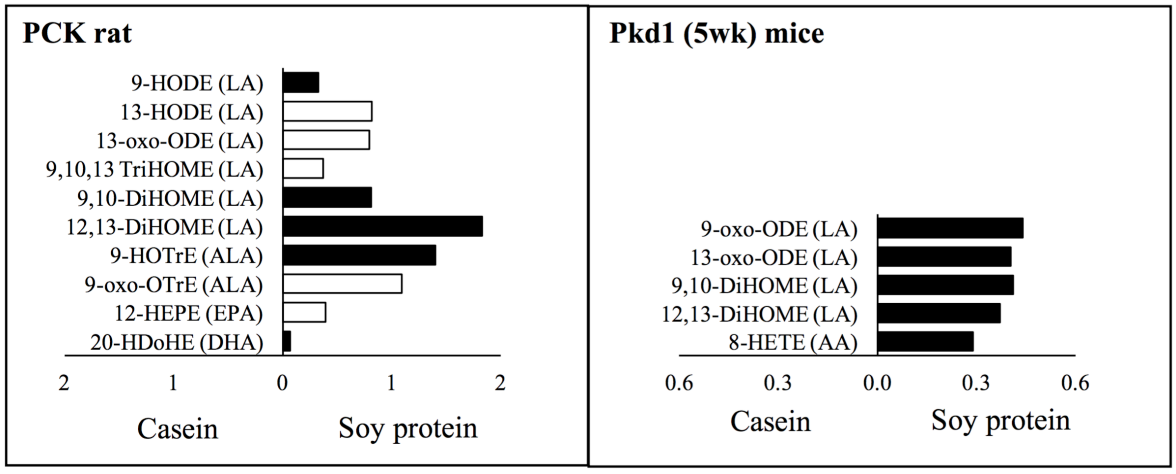
Other n-6 fatty acid derived oxylipins (1 from gamma-linolenic acid (GLA), 2 from dihomo-gamma-linolenic acid (DGLA), 3 non-enzymatic AA products) had similar patterns to the LA and AA oxylipins. In general, they were lowest in the fish oil groups, with levels being intermediate in the flax oil groups (Tables 7.2-7.9).

Figure 7.1: Summary of dietary oil effects on the relative abundance of total renal oxylipins derived from individual PUFA



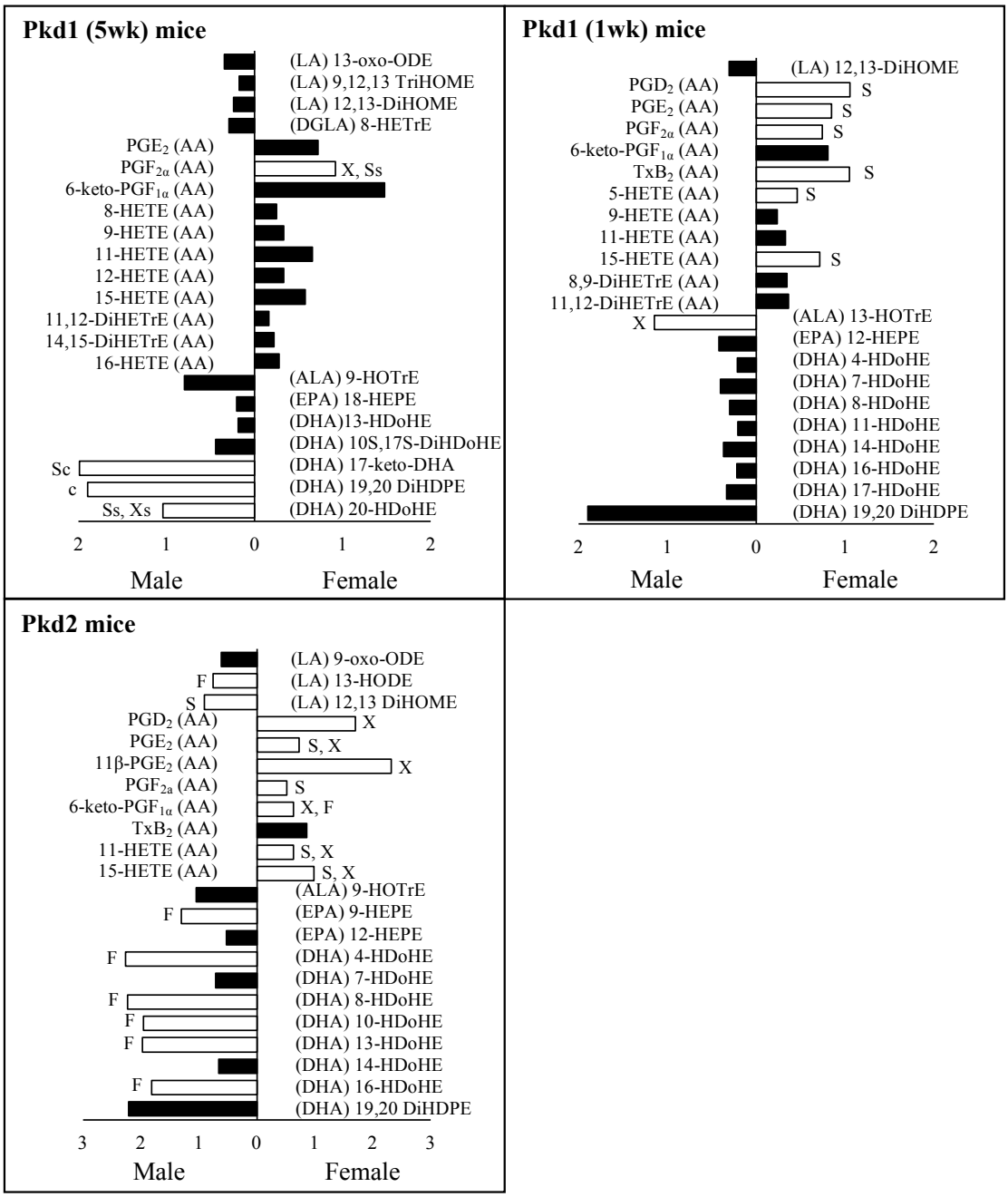
Effect of dietary oil on total renal oxylipins from each PUFA. Darker/lighter color indicates higher/lower levels, respectively, in flax and fish oil groups compared to soy oil group.

Figure 7.2: Summary of dietary protein effects on the relative levels of renal oxylipins



Relative differences in renal oxylipins that displayed protein effects are presented, calculated by comparing the mean oxylipin concentration in the soy protein diet to the mean concentration in the casein diet. Precursor fatty acids are shown in parentheses. Black bars indicate oxylipins with overall protein effects and open bars indicate protein effects only in the flax oil subgroup

Figure 7.3: Summary of sex effects on the relative levels of renal oxylipins



Relative differences in renal oxylipns that displayed sex effects are presented as fold difference values, calculated by comparing the mean oxylipin concentration in the sex that is higher to the mean concentration in the other. Oxylipins with overall sex effect are indicated by black bars and those with subgroup effects are shown with open bars with subgroups indicated as follows: S, soy oil; X, flax oil; F, fish oil; c, casein; s, soy protein.

Table 7.2: The effect of sex, dietary oil and protein sources on renal oxylipin totals from each PUFA in Pkd1 (5wk) mice (pg/mg dry tissue)

Total Oxylipins	Female						Male						Effects	
	Casein			Soy protein			Casein			Soy protein				
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil		
LA oxylipins (8)	18071 ± 1897	13963 ± 953	5723 ± 794	21109 ± 1523	13700 ± 1447	8643 ± 1247	18032 ± 1261	12296 ± 1543	5797 ± 884	23879 ± 4067 ^{A}	18913 ± 2617 ^{B}	7600 ± 1398 ^{C}	P	O
DGLA oxylipins (2)	265 ± 21.3	286 ± 16.0	117 ± 13.0	259 ± 22.4	226 ± 22.0	150 ± 11.0	339 ± 35.9	242 ± 33.3	136 ± 15.4	338 ± 52.8 ^{A}	260 ± 28.3 ^{B}	149 ± 20.9 ^{C}		O
AA oxylipins (16)	11924 ± 705	7161 ± 681	4495 ± 437	11729 ± 994	7606 ± 1358	6477 ± 980	9626 ± 846	4590 ± 762	4091 ± 493	10218 ± 1481 ^{A}	5216 ± 944 ^{B}	4665 ± 700 ^{C}	S	O
ALA oxylipins (3)	29461 ± 4459 ^{cd}	82137 ± 18945^{abc}	9433 ± 1535 ^{d}	28313 ± 4193 ^{dc}	98516 ± 24114 ^{ab}	11378 ± 3418 ^{d}	40569 ± 8697 ^{bcd}	71397 ± 23663 ^{bcd}	8540 ± 1397 ^{d}	38646 ± 6732 ^{bcd}	146940 ± 27343^{a}	12232 ± 3382 ^{d}	Int	
EPA oxylipins (7)	2390 ± 508	18091 ± 2291	27654 ± 2130	1984 ± 178	13207 ± 1459	35515 ± 5969	2998 ± 336	19591 ± 949	25365 ± 4202	2732 ± 476 ^{C}	16070 ± 4685 ^{B}	30659 ± 4610 ^{A}		O
DHA oxylipins (12)	15061 ± 1703 ^{c}	21056 ± 2985 ^{bc}	41600 ± 5659 ^{ab}	13936 ± 1609 ^{c}	14634 ± 2120 ^{c}	44228 ± 3098 ^{ab}	24106 ± 1599 ^{bc}	30422 ± 4771 ^{bc}	33787 ± 5686 ^{bc}	26245 ± 2114 ^{bc}	28306 ± 3016 ^{bc}	64044 ± 12612 ^{a}	Int	

All values represent mean ± SEM (n=5-7). The number of individual oxylipins in each total is in parenthesis. S, sex effect; P, protein effect; O, oil effect; Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male soy protein groups) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.3: The effect of dietary oil and sex on renal oxylipin totals from each PUFA in Pkd1 (1wk) mice (pg/mg dry tissue)

Total Oxylipins	Male			Female			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
LA oxylipins (8)	18150 ± 1858^{A}	14823 ± 1204^{B}	8027± 887^{C}	20333 ± 1377	12178 ± 1903	7927 ± 515	O
DGLA oxylipins (2)	225 ± 25.7^{A}	187 ± 11.3^{B}	128 ± 13.0^{C}	247 ± 23.9	194 ± 11.3	100 ± 3.28	O
AA oxylipins (17)	8445 ± 533^{A}	4437 ± 352^{B}	4400 ± 416^{B}	11455 ± 732	5270 ± 306	5028 ± 478	S O
ALA oxylipins (3)	14393 ± 2816^{b}	79021 ± 18446^{a}	7106 ± 882^{b}	13877 ± 1056^{b}	28840 ± 14863^{ab}	8957 ± 1074^{b}	Int
EPA oxylipins (8)	2500 ± 336 ^{c}	21200 ± 4247^{b}	43289 ± 3809^{a}	2830 ± 107^{c}	22330 ± 592^{b}	36575 ± 1105^{ab}	Int
DHA oxylipins (14)	25053 ± 2225^{B}	34444 ± 3299^{B}	50994 ± 5584^{A}	24813 ± 2157	25147 ± 2388	37566 ± 2469	S O

All values represent mean ± SEM (n=5-7). The number of individual oxylipins in each total is in parenthesis. S, Sex effect; O, oil effect; Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.4: The effect of dietary oil and sex on renal oxylipin totals from each PUFA in Pkd2 mice (pg/mg dry tissue)

Total Oxylipins	Male			Female			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
LA oxylipins (8)	17834 ± 2292^{A}	9142 ± 1993^{B}	7116 ± 594^{C}	16121 ± 2484	10653 ± 1627	4599 ± 512	O
DGLA oxylipins (2)	173 ± 21.4^{A}	112 ± 31.2^{B}	116 ± 15.8^{B}	177 ± 26.7	108 ± 24.2	58.1 ± 18.6	O
AA oxylipins (18)	6861 ±1042^{A}	2266±355^{B}	3644 ±575^{B}	9359 ±1084	4344 ± 613	2546 ± 611	O
ALA oxylipins (2)	13833 ± 2947^{bc}	56719 ± 19057^{a}	5449 ± 1067^{c}	10894 ± 4185^{bc}	30586 ± 7269^{b}	3073 ± 2097^{c}	Int
EPA oxylipins (7)	1702 ± 489^{C}	7171 ± 1430^{B}	14730 ± 2210^{A}	1222 ± 256	5924 ± 1046	9837 ± 2758	O
DHA oxylipins (12)	24504 ± 8442	15904 ± 3939	42472 ± 7947	22491 ± 3129	14081 ± 4252	20066 ± 10547	

All values represent mean ± SEM (n=4-8). The number of individual oxylipins in each total is in parenthesis. O, oil effect; Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.5: The effect of dietary oil and protein sources on renal oxylipin totals from each PUFA in PCK rats (pg/mg dry tissue)

Total Oxylipins	Casein			Soy protein			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
LA oxylipins (8)	42566 ± 3997	25497 ± 4356	18651 ± 2466	51332 ± 3192^{A}	44091 ± 3201^{B}	20231 ± 2856^{C}	P O
DGLA oxylipins (2)	807 ± 34.5^{a}	365 ± 75.5^{c}	441 ± 34.0^{c}	761 ± 117^{ab}	479 ± 50.4^{bc}	438 ± 60.0^{c}	Int
AA oxylipins (19)	26241 ± 1066^{a}	11208 ± 1972^{c}	10519 ± 1146^{c}	18860 ± 3054^{ab}	13350 ± 1350^{bc}	9891 ± 884^{c}	Int
ALA oxylipins (3)	91407 ± 11133	291749 ± 101228	44468 ± 7778	118394 ± 17928^{B}	541357 ± 103304^{A}	36194 ± 5090^{C}	O
EPA oxylipins (6)	4614 ± 364^{b}	11620 ± 1798^{b}	39856 ± 3120^{a}	4393 ± 602^{b}	14510 ± 601^{b}	31878 ± 4911^{a}	Int
DHA oxylipins (10)	10736 ± 579^{b}	6466 ± 851^{b}	34136 ± 3245^{a}	10197 ± 1443^{b}	9650 ± 429^{b}	29708 ± 4535^{a}	Int

All values represent mean ± SEM (n=6-8). The number of individual oxylipins in each total is in parenthesis. P, protein effect; O, oil effect; Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in soy protein groups) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.6: Dietary oil, protein and sex effects on renal oxylipins in Pkd1 (5wk) mice (pg/mg dry tissue)

Oxylipin	Male						Female								
	Casein			Soy protein			Casein			Soy protein					
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects		
n-6 oxylipins															
LA															
9-HODE	4451 ± 270	3119 ± 352	1306 ± 223	5654± 1111^{A}	3495 ± 460^{B}	2085 ± 534^{C}	4137± 560	3068 ± 307	1164 ± 132	4840± 482	2854 ± 277	1542 ± 170			O
9-oxo-ODE	1840 ± 195	1491 ± 249	688 ± 184	3330 ± 702^{A}	2585 ± 690^{B}	637± 86.2^{C}	2531± 304	1866 ± 255	755 ± 165	3212± 421	2129 ± 360	1316 ± 274		P	O
13-HODE	5097 ± 692	2943 ± 351	1334 ± 172	5773 ± 1120^{A}	4016 ± 444^{B}	2018 ± 573^{C}	5256± 586	3608 ± 260	1239 ± 151	5456± 439	3215 ± 182	1916 ± 183			O
13-oxo-ODE	2856 ± 336	3040 ± 555	905 ± 177	5174 ± 1032^{A}	3520 ± 660^{A}	1592 ± 575^{B}	2212± 438	2254 ± 391	1125 ± 208	3203± 484	2278 ± 673	1641 ± 410	S	P	O
9,10,13 TriHOME	3243 ± 518	2862 ± 252	1100 ± 149	3365 ± 508^{A}	3863 ± 645^{A}	1078 ± 127^{B}	2869± 330	2283 ± 208	1027 ± 184	3111 ± 340	2328 ± 255	1569 ± 214			O
9,12,13 TriHOME	1142 ± 161	989 ± 100	411 ± 60.5	1132 ± 160^{A}	1295 ± 193^{A}	388 ± 44.3^{B}	946 ± 124	793 ± 83.2	369 ± 56.9	1126± 76.4	767 ± 66.4	563 ± 78.0	S		O
9,10-DiHOME	94.6 ± 10.90	68.6 ± 4.60	30.6 ± 5.00	119 ± 17.2^{A}	84.3 ± 6.60^{B}	60.1 ± 12.6^{C}	75.2 ± 8.10	53.9 ± 4.70	30.2 ± 1.80	101 ± 4.50	76.7 ± 6.40	57.2 ± 5.50		P	O
12,13-DiHOME	61.3 ± 7.60	51.8 ± 7.30	23.4 ± 4.20	80.9 ± 10.5^{A}	55.2 ± 3.10^{B}	42.2 ± 6.20^{C}	45.8 ± 3.10	37.3 ± 4.50	20.5 ± 1.00	59.7 ± 5.90	52.5 ± 4.10	38.7 ± 5.60	S	P	O
DGLA															
8-HETrE	265 ± 29.4	181 ± 19.9	94.4 ± 10.7	242 ± 35.9^{A}	177 ± 19.1^{B}	105 ± 14.3^{C}	152 ± 15.5	187 ± 16.8	79.4 ± 10.2	170 ± 17.4	141 ± 17.4	94.7 ± 4.20	S		O
15-HETrE	88.7 ± 20.6	61.0 ± 14.5	41.9 ± 5.50	96.7 ± 18.6^{A}	83.0 ± 11.5^{A}	43.9 ± 9.00^{B}	113 ± 15.5	98.4 ± 14.4	37.2 ± 3.60	89.3 ± 15.4	85.3 ± 12.8	55.8 ± 9.60			O
AA															
PGD_2	166 ± 33.3	117 ± 18.6	117 ± 34.7	178 ± 38.3^{A}	103 ± 29.1^{B}	120 ± 25.7^{B}	203 ± 31.5	87.8 ± 15.9	91.7 ± 14.0	204 ± 25.1	118 ± 23.0	108 ± 17.4			O
PGE_2	280 ± 24.8	112 ± 13.3	50.7 ± 3.70	229 ± 21.3^{A}	111 ± 18.5^{B}	87.2 ± 18.5^{C}	419 ± 17.6	191 ± 32.6	73.1 ± 5.70	434 ± 46.8	282 ± 53.2	96.3 ± 11.0	S		O
$PGF_{2\alpha}$	218 ± 25.9^{ab}	89.9 ± 6.60^{d}	61.9 ± 5.00^{d}	170 ± 23.2^{bc}	104 ± 15.2^{cd}	78.7 ± 7.80^{d}	354 ± 29.3^{a}	165 ± 13.2^{bc}	76.3 ± 7.90^{d}	348 ± 34.2^{a}	184 ± 25.2^{b}	100 ± 6.50^{cd}		Int	
6-keto-$PGF_{1\alpha}$	303 ± 50.4	131 ± 15.1	75.2 ± 8.20	244 ± 37.8^{A}	146 ± 24.0^{B}	80.5 ± 12.9^{C}	760 ± 122	271 ± 32.4	93.7 ± 9.30	727 ± 92.8	433 ± 142	138 ± 9.80	S		O
5-HETE	1828 ± 173	765 ± 74.5	767 ± 91.9	1675 ± 190^{A}	779 ± 112^{B}	898 ± 115^{B}	1740± 197	1128 ± 140	770 ± 53.4	1726± 100	1052 ± 298	1016 ± 86.0			O
5-oxo-ETE	411 ± 50.5	197 ± 29.1	200 ± 34.1	357 ± 37.5^{A}	204 ± 27.6^{B}	149 ± 21.7^{B}	390 ± 53.4	252 ± 35.9	243 ± 27.4	393 ± 56.0	153 ± 16.5	252 ± 16.5			O
8-HETE	445 ± 78.4	187 ± 67.8	190 ± 29.8	504 ± 122^{A}	266 ± 57.6^{B}	241 ± 60.8^{B}	424 ± 37.8	377 ± 60.5	179 ± 22.3	596 ± 136	404 ± 95.4	312 ± 31.8	S	P	O
9-HETE	818 ± 162	379 ± 127	398 ± 60.3	655 ± 148^{A}	357 ± 56.6^{B}	573 ± 173^{B}	1177 ± 110	619 ± 75.0	411 ± 62.9	848 ± 112	549 ± 122	635 ± 163	S		O
11-HETE	798 ± 172	357 ± 70.4	318 ± 40.7	937 ± 190^{A}	419 ± 82.7^{B}	361 ± 83.2^{C}	1299± 100	815 ± 119	347 ± 37.5	1324± 97.9	907 ± 212	598 ± 101	S		O
12-HETE	1270 ± 284.	367 ± 139	322 ± 39.0	1187 ± 368^{A}	674 ± 208^{B}	326 ± 68.0^{C}	1657± 269	791 ± 110	270 ± 29.5	1165± 178	1038 ± 280	592 ± 93.5	S		O
15-HETE	1428 ± 206	564 ± 87.1	516 ± 57.9	1297 ± 182^{A}	613 ± 100^{B}	598 ± 110^{C}	2008 ± 137	1051 ± 103	625 ± 46.7	1958 ± 139	1376 ± 383	874 ± 119	S		O
15-oxo-ETE	1556 ± 282	1175 ± 186	923 ± 184	2497 ± 372^{A}	1242 ± 304^{B}	1007 ± 164^{B}	1519± 329	1285 ± 224	1124 ± 186	1572± 244	1032 ± 184	1489 ± 414			O
5,6-DiHETrE	126 ± 12.7	57.5 ± 6.50	65.1 ± 10.0	130 ± 14.6^{A}	58.0 ± 8.90^{B}	68.1 ± 12.4^{B}	96.6 ± 15.6	69.7 ± 7.80	52.1 ± 8.70	115 ± 12.3	61.2 ± 16.0	77.7 ± 5.30			O

8,9-DiHETrE	17.3 ± 1.80	10.9 ± 1.80	9.50 ± 2.20	14.3 ± 2.50^{A}	7.60 ± 1.60^{B}	9.60 ± 2.60^{B}	17.3 ± 2.10	8.50 ± 2.00	10.5 ± 2.00	17.2 ± 1.60	10.0 ± 0.50	12.2 ± 0.40		O
11,12-DiHETrE	41.7 ± 6.30	16.0 ± 2.30	18.7 ± 3.10	34.9 ± 5.00^{A}	24.1 ± 4.20^{B}	16.1 ± 1.40^{B}	46.8 ± 5.40	23.2 ± 1.90	20.2 ± 1.00	39.6 ± 3.40	21.4 ± 1.10	24.8 ± 1.80	S	O
14,15-DiHETrE	50.4 ± 6.20	21.4 ± 3.20	18.9 ± 2.80	43.9 ± 5.20^{A}	24.5 ± 2.80^{B}	20.5 ± 3.00^{B}	59.0 ± 4.70	28.4 ± 2.40	22.7 ± 2.40	47.3 ± 6.10	32.1 ± 7.70	30.0 ± 3.40	S	O
16-HETE	184 ± 17.8	75.2 ± 14.9	64.2 ± 13.4	176 ± 19.4^{A}	85.7 ± 12.6^{B}	65.5 ± 6.40^{B}	183 ± 13.8	102 ± 12.7	86.8 ± 12.4	215 ± 22.9	125 ± 13.7	121 ± 24.4	S	O
n-3 oxylipins														
ALA														
9-HOTrE	528 ± 76.9	2717 ± 488	101 ± 28.9	518 ± 88.0^{B}	1905 ± 307^{A}	160 ± 41.4^{C}	303 ± 59.0	1419 ± 285	103 ± 17.9	294 ± 32.2	1085 ± 263	96.8 ± 15.3	S	O
9-oxo-OTrE	189 ± 21.3	490 ± 122	40.2 ± 11.4	421 ± 93.7^{B}	707 ± 158^{A}	86.0 ± 46.2^{C}	266 ± 90.6	534 ± 131	119 ± 58.2	282 ± 66.3	511 ± 198	115 ± 45.1		O
13-HOTrE	39852± 8629	10240± 16508	8399 ± 1362	37707± 6567^{B}	14432± 26919^{A}	1201± 3320^{C}	2889± 4349	96328± 12726	9262 ± 1484	2773± 4112	9700± 23874	11189± 3372		O
EPA														
5-HEPE	382 ± 59.8	3505 ± 412	4601 ± 1086	400 ± 54.7^{C}	1876 ± 316^{B}	4372 ± 559^{A}	300 ± 106	2688 ± 535	4739 ± 485	265 ± 35.7	1411 ± 238	5077 ± 863		O
8-HEPE	79.8 ± 9.40	461 ± 112	374 ± 99.5	85.3 ± 21.0^{B}	408 ± 72.4^{A}	385 ± 93.6^{A}	72.5 ± 26.1	459 ± 92.6	454 ± 37.7	92.4 ± 17.5	359 ± 109	744 ± 173		O
9-HEPE	667 ± 130	3805 ± 702	4157 ± 1169	667 ± 123^{C}	2804 ± 860^{B}	3873 ± 689^{A}	519 ± 178	3902 ± 806	4377 ± 468	512 ± 94.7	2557 ± 527	6789 ± 1285		O
11-HEPE	237 ± 34.4	1283 ± 348	1253 ± 471	267 ± 85.2^{B}	1113 ± 417^{A}	1244 ± 261^{A}	215 ± 59.0	1700 ± 395	1608 ± 131	217 ± 42.9	1319 ± 257	2850 ± 850		O
12-HEPE	761 ± 88.3^{b}	2421 ± 504^{a}	2342 ± 360^{a}	574 ± 120^{b}	4085 ± 1632^{a}	2350 ± 350^{a}	509 ± 126^{b}	3777 ± 630^{a}	2647 ± 344^{a}	337 ± 45.0^{b}	2911 ± 474^{a}	5973 ± 1319^{a}		Int
15-HEPE	285 ± 52.4	790 ± 262	1131 ± 234	252 ± 46.0^{C}	885 ± 306^{B}	1040 ± 149^{A}	300 ± 50.3	881 ± 194	1405 ± 171	196 ± 20.6	1100 ± 74.9	1723 ± 609		O
18-HEPE	586 ± 40.2	7730 ± 1724	11976± 2449	501 ± 99.3^{C}	4981 ± 1258^{B}	1739± 4552^{A}	499 ± 39.2	4831 ± 609	1242± 1348	381 ± 59.2	3549 ± 573	12360 ± 2111	S	O
DHA														
4-HDoHE	2330 ± 184^{bcd}	2352 ± 168^{bcd}	2563 ± 437^{abcd}	2317 ± 197^{bcd}	2143 ± 217^{bcde}	3917 ± 399^{a}	1342± 219de	1935 ± 331^{cde}	2649 ± 150^{abc}	1078± 65.1^{de}	961 ± 132^{e}	3417 ± 424^{ab}		Int
7-HDoHE	179 ± 38.4	203 ± 42.7	327 ± 77.9	252 ± 58.5^{B}	221 ± 49.7^{B}	297 ± 44.0^{A}	145 ± 18.8	197 ± 38.6	260 ± 22.8	171 ± 40.3	134 ± 23.6	426 ± 71.5		O
10-HDoHE	158 ± 23.2	281 ± 47.7	259 ± 62.8	247 ± 67.4^{B}	188 ± 42.6^{B}	335 ± 84.9^{A}	139 ± 27.3	241 ± 58.6	220 ± 12.8	217 ± 81.2	158 ± 14.7	381 ± 62.1		O
10S,17S-DiHDoHE	68.3 ± 6.50	57.6 ± 13.6	64.0 ± 16.4	51.8 ± 16.5^{B}	60.0 ± 10.1^{B}	107 ± 41.9^{A}	30.9 ± 5.70	57.0 ± 12.8	52.7 ± 7.00	36.6 ± 13.8	25.8 ± 3.00	80.7 ± 15.2	S	O
11-HDoHE	419 ± 86.6	545 ± 102	665 ± 130	636 ± 165^{B}	558 ± 154 ^{B}	820 ± 207^{A}	391 ± 56.1	605 ± 108	641 ± 42.1	387 ± 58.2	379 ± 29.0	1107 ± 200		O
13-HDoHE	185 ± 26.6	180 ± 19.7	247 ± 46.7	189 ± 23.1^{B}	166 ± 29.3^{B}	301 ± 59.4^{A}	141 ± 13.2	168 ± 21.5	227 ± 11.3	123 ± 12.0	120 ± 12.1	290 ± 36.9	S	O
14-HDoHE	792 ± 94.6	716 ± 142	905 ± 194	871 ± 181^{B}	814 ± 200^{B}	1053 ± 226^{A}	646 ± 87.5	759 ± 109	795 ± 49.4	583 ± 117.2	605 ± 71.9	1215 ± 211		O
16-HDoHE	846 ± 43.9^{abcd}	836 ± 60.1^{abcd}	984 ± 201^{abc}	865 ± 82.3^{abc}	877 ± 117^{abcd}	1453 ± 194^{a}	565 ± 59.6^{cd}	711 ± 105^{bcd}	1106 ± 68.5^{ab}	526 ± 60.1^{cd}	493 ± 46.3^{d}	1273 ± 190^{a}		Int
17-HDoHE	2431 ± 107^{abcd}	2587 ± 207^{abc}	3013 ± 453^{abc}	2660 ± 261^{abc}	2499 ± 302^{abcd}	4640 ± 757^{a}	1666± 181^{cd}	2252 ± 437^{bcd}	3232 ± 147^{ab}	1599± 233^{cd}	1440 ± 197^{d}	4186 ± 627^{a}		Int
17-keto-DHA	3025 ± 857^{abc}	3028 ± 598^{abc}	3673 ± 828^{ab}	2976 ± 534^{abc}	1685 ± 238^{bcd}	3109 ± 272^{ab}	1013± 141^{d}	1137 ± 87.3^{cd}	2832 ± 318^{ab}	1673± 526^{bcd}	1230 ± 165^{cd}	4440 ± 847^{a}		Int
19,20 DiHDPE	454 ± 59.7^{ab}	512 ± 49.6^{a}	471 ± 109^{ab}	360 ± 43.3^{abc}	359 ± 45.2^{abc}	375 ± 39.6^{abc}	150 ± 9.7^{de}	136 ± 15.0^{de}	210 ± 11.7^{cd}	94.0 ± 12.4^{e}	114 ± 24.1^{e}	234 ± 28.2^{bcd}		Int
20-HDoHE	14926± 1548^{cde}	22950± 2683^{abcd}	29219± 3625^{abc}	14821± 1213^{cde}	18737± 2279^{bcd}	4817± 12146^{a}	8832± 1018^{ef}	13104± 2125^{def}	3427± 2459^{ab}	7450± 856^{f}	8974 ± 1532^{ef}	27179± 1093^{abc}		Int

All values represent mean ± SEM (n=3-7). S, sex effect; P, protein effect; O, oil effect, Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male soy protein groups) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.7: Dietary oil and sex effects on renal oxylipins in Pkd1 (1wk) mice (pg/mg dry tissue)

Oxylipin	Male			Female			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
n-6 oxylipins							
LA							
9-HODE	4037 ± 385^{A}	3199 ± 264^{B}	1432 ± 133^{C}	4201 ± 256	2753 ± 355	1473 ± 155	O
9-oxo-ODE	1769 ± 302^{A}	1571 ± 175^{AB}	1123 ±195^{B}	1973 ± 243	1050 ± 286	931 ± 8.50	O
13-HODE	4520 ± 459^{A}	3476 ± 678^{B}	1995 ±323^{C}	5265 ± 610	3087 ± 364	1787 ± 162	O
13-oxo-ODE	4036 ± 531^{A}	4126 ± 674^{A}	2251 ± 306^{B}	5939 ± 783	3128 ±1108	2101 ± 165	O
9,10,13 TriHOME	1523 ± 102^{ab}	1660 ± 131^{ab}	904 ± 125^{c}	1892 ± 222^{a}	1096±184^{bc}	756 ± 40.4^{c}	Int
9,12,13 TriHOME	526 ± 48.1^{A}	507 ± 35.3^{A}	301 ± 34.4^{B}	611 ± 88.8	384 ± 59.2	222 ± 19.3	O
9,10-DiHOME	83.9 ± 10.0^{A}	79.3 ± 8.50^{A}	46.4 ± 7.70^{B}	82.0 ± 5.30	63.7 ± 11.2	35.6 ± 2.60	O
12,13-DiHOME	1655 ± 219^{A}	1441 ± 128^{A}	834 ± 105^{B}	1421 ± 70.0	966 ± 165	622 ± 30.6	S O
GLA							
13-HOTrE-γ	50.9 ± 6.00^{A}	21.5 ± 3.20^{B}	15.0 ± 2.70^{B}	50.0 ± 7.30	21.9 ± 2.70	10.1 ± 1.40	O
DGLA							
8-HETrE	163 ± 8.00^{A}	119.3 ± 8.4^{B}	78.9 ± 8.90^{C}	164 ± 14.0	125 ± 8.80	67.2 ± 2.80	O
15-HETrE	89.8 ± 7.20^{A}	68.2 ± 3.90^{B}	49.4 ± 5.10^{C}	82.9 ± 10.2	69.7 ± 8.20	32.8 ± 2.20	O
AA							
PGD_2	518 ± 62.1^{b}	245 ± 29.1^{cd}	166 ± 19.6^{d}	1064 ±78.2^{a}	410 ±12.3^{bc}	257±32.7^{cd}	Int
PGE_2	261 ± 22.7^{b}	94.3 ± 11.0^{c}	70.6 ± 9.56^{c}	482 ± 30.9^{a}	151 ± 22.2^{c}	74.9 ±14.4^{c}	Int
$PGF_{2\alpha}$	240 ± 19.5^{b}	147 ± 23.2^{bc}	89.9 ± 12.2^{c}	419 ± 42.9^{a}	200 ±59.7^{bc}	117 ± 14.4^{c}	Int
6-k-$PGF_{1\alpha}$	418 ± 26.7^{A}	279 ± 51.2^{B}	167.7 ± 21.6^{C}	976 ± 54.5	341 ± 8.0	246 ± 33.7	S O
TxB_2	28.7 ± 4.90^{b}	22.3 ± 2.70^{bc}	12.8 ± 1.26^{c}	58.8 ± 8.00^{a}	28.6±1.30^{bc}	17.5±2.10^{bc}	Int
5-HETE	1830 ± 176^{b}	881 ± 45.5^{c}	982 ± 94.9^{c}	2669 ± 244^{a}	984 ± 53.2^{c}	1115±55.1^{c}	Int
5-oxo-ETE	295 ± 38.6^{ab}	159 ± 19.9^{c}	286 ± 53.7^{bc}	558 ± 80.1^{a}	214 ±30.4^{bc}	187 ±26.9^{bc}	Int
8-HETE	847 ± 69.0^{A}	489 ± 79.1^{B}	420 ± 61.2^{B}	913 ± 175	351 ± 33.7	596 ± 187	O
9-HETE	1213 ± 127^{A}	602 ± 59.2^{B}	707 ± 67.4^{B}	1603 ± 71.8	763 ± 88.2	758 ± 39.3	S O
11-HETE	626 ± 45.1^{A}	394 ± 45.1^{B}	418 ± 59.7^{B}	945 ± 197	454 ± 34.3	509 ± 30.2	S O
12-HETE	1150 ± 83.0^{A}	675 ± 53.7^{B}	532 ± 40.9^{B}	1332 ± 127	704 ± 180	480 ± 106	O
15-HETE	831 ± 60.0^{b}	438 ± 35.0^{c}	430 ± 54.0^{c}	1427 ± 131^{a}	565 ±43.8^{bc}	552±11.2^{bc}	Int
5,6-DiHETrE	61.3 ± 5.30^{A}	27.0 ± 1.80^{B}	26.4 ± 3.80^{B}	66.9 ± 10.8	17.5 ± 0.70	25.5 ± 5.20	O
8,9-DiHETrE	13.4 ± 1.10^{A}	7.0 ± 0.80^{B}	6.70 ± 1.20^{B}	18.8 ± 2.60	11.1 ± 3.40	6.50 ± 1.30	S O
11,12-DiHETrE	34.4 ± 3.30^{A}	19.7 ± 1.80^{B}	20.1 ± 1.90^{B}	49.3 ± 4.60	26.6 ± 5.10	25.1 ± 1.80	S O
14,15-DiHETrE	38.1 ± 2.00^{A}	21.7 ± 1.60^{B}	22.7 ± 2.10^{B}	44.7 ± 4.10	17.9 ± 2.50	18.7 ± 1.00	O
16-HETE	49.5 ± 5.00^{A}	44.4 ± 4.90^{B}	42.5 ± 2.80^{B}	59.8 ± 3.00	32.7 ± 0.90	44.2 ± 6.10	O
n-3 oxylipins							
ALA							
9-HOTrE	277 ± 75.1^{B}	1474 ± 81.7^{A}	108 ± 19.8^{C}	236 ± 38.8	648 ± 40.6	90.6 ± 11.0	O
9-oxo-OTrE	138 ± 42.9^{B}	654 ± 116^{A}	124 ± 64.9^{C}	256 ± 145	394 ± 132	62.8 ± 27.7	O
13-HOTrE	13979±2715^{b}	90309±15656^{a}	6875 ±822^{b}	13385±1153^{b}	42021±6624^{b}	8804±1102^{b}	Int

EPA								
PGE_3	8.50 ± 2.90^{B}	62.9 ± 7.20^{A}	99.1 ± 17.2^{A}	11.1 ± 1.60	65.4 ± 5.90	96.0 ± 7.00		O
5-HEPE	376 ± 36.2^{C}	3126 ± 466^{B}	5241 ± 605^{A}	401 ± 32.9	3069 ± 282	5698±628.8		O
8-HEPE	76.5 ± 14.2^{C}	498 ± 98.9^{B}	885 ± 192^{A}	64.1 ± 10.8	376 ± 43.1	924 ± 277		O
9-HEPE	252 ± 20.6^{C}	2338 ± 320^{B}	4125 ± 672^{A}	294 ± 35.4	1951 ± 195	3541 ± 140		O
11-HEPE	122 ± 14.0^{C}	1115 ± 147^{B}	1723 ± 272^{A}	149 ± 25.7	957 ± 94.5	1511 ± 46.3		O
12-HEPE	442 ± 49.6^{C}	4191 ± 419^{B}	5928 ± 485^{A}	399 ± 25.4	3437 ± 484	3601 ± 541	S	O
15-HEPE	72.1 ± 13.8^{C}	551 ± 82.8^{B}	977 ± 102^{A}	81.6 ± 11.7	559 ± 46.2	878 ± 45.8		O
18-HEPE	1382 ± 158^{C}	12924±1606^{B}	25060±2460^{A}	1446 ± 122	11917 ±150	20327±1440		O
DHA								
4-HDoHE	1098 ± 115^{B}	1316 ± 138^{B}	1903 ± 149^{A}	1076 ± 129	987 ± 117	1490 ± 185	S	O
7-HDoHE	203 ± 25.6^{B}	283 ± 40.2^{B}	398 ± 67.9^{A}	190 ± 13.6	151 ± 8.10	288 ± 45.8	S	O
8-HDoHE	988 ± 123^{B}	1320 ± 117^{B}	1946 ± 197^{A}	896 ± 116	903 ± 75.3	1473 ± 185	S	O
10-HDoHE	136 ± 10.3^{B}	179 ± 11.2^{B}	251 ± 35.5^{A}	145 ± 21.1	138 ± 12.5	220 ± 16.0		O
10S,17S-DiHDoH	29.6 ± 4.30	32.2 ± 4.50	47.6 ± 9.70	29.4 ± 6.00	21.7 ± 4.80	39.7 ± 7.70		
11-HDoHE	238 ± 29.2^{C}	330 ± 16.6^{B}	533 ± 44.4^{A}	219 ± 15.9	271 ± 31.3	422 ± 43.7	S	O
13-HDoHE	82.4 ± 5.50^{B}	111 ± 11.5^{B}	152 ± 15.3^{A}	97.5 ± 4.40	74.0 ± 5.30	120 ± 9.30		O
14-HDoHE	511 ± 45.1^{B}	723 ± 69.9^{B}	974 ± 93.1^{A}	505 ± 40.3	486 ± 59.4	624 ± 55.6	S	O
16-HDoHE	454 ± 35.3^{B}	583 ± 52.9^{B}	746 ± 72.4^{A}	479 ± 38.6	365 ± 30.0	622 ± 39.4	S	O
17-HDoHE	969 ± 97.2^{B}	1238 ± 126^{B}	1870 ± 243^{A}	1001 ± 70.3	751 ± 36.5	1301 ± 126	S	O
17-keto-DHA	481 ± 43.4^{B}	535 ± 65.8^{B}	988 ± 123^{A}	622 ± 68.8	501 ± 54.5	877 ± 178		O
Resolvin D2	102 ± 11.8^{B}	143 ± 20.3^{B}	202 ± 13.1^{A}	93.2 ± 17.2	82.1 ± 14.9	189 ± 38.7		O
19,20 DiHDPE	222.3 ± 22.6^{B}	313 ± 20.6^{A}	352 ± 39.2^{A}	94.1 ± 10.7	81.5 ± 6.10	131 ± 10.0	S	O
20-HDoHE	21038 ± 1899^{B}	29300 ± 2918^{B}	44561 ± 5475^{A}	20938±1975	21761±2117	32162±1991		O
Non-enzymatic AA oxylipins								
8-iso $PGF_{2\alpha}$III	621 ± 50.7^{b}	342 ± 64.6^{c}	243 ± 30.2^{c}	1030 ± 68.3^{a}	527 ± 134^{bc}	243 ± 64.8^{c}		Int

All values represent mean ± SEM (n=3-7). S, sex effect; O, oil effect, Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.8: Dietary oil and sex effect son renal oxylipins in Pkd2 mice (pg/mg dry tissue)

Oxylipin	Male			Female			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
n-6 oxylipins							
LA							
9-HODE	4529±666^{A}	2079±482^{B}	1554±182^{C}	4454±1048	3087±563	1047±79.5	O
9-oxo-ODE	1220±170^{A}	785±190^{B}	712±158^{B}	788±127	485±70.1	412±167	S O
13-HODE	6168±888^{a}	2803±572^{cd}	2059±194^{d}	5003±910^{ab}	3448±562^{bc}	1172±95.5^{e}	Int
13-oxo-ODE	3019±579^{A}	1467±362^{B}	1135±141^{B}	2247±526	1475±467	839±189	O
9,10,13 TriHOME	2127±402^{A}	1265±293^{B}	1020±147^{B}	24430±350	1622±347	690±174	O
9,12,13 TriHOME	1167±199^{A}	638±149^{B}	522±72.8^{B}	1028±189	438±61.0	357±51.8	O
9,10 DiHOME	122±21.7^{A}	52.7±10.0^{B}	57.3±7.60^{B}	82.5±21.7	84.4±17.8	39.2±3.80	O
12,13 DiHOME	146±16.4^{a}	53.9±10.7^{b}	56.8±5.80^{b}	76.6±21.6^{b}	82.3±14.6^{b}	42.1±2.10^{b}	Int
DGLA							
8-HETrE	119±32.3^{A}	48.9±14.4^{B}	50.9±6.40^{B}	96.7±14.6	54.3±16.0	18.3±6.8	O
15-HETrE	76.5±5.30	63.2±17.0	64.9±10.7	80.6±16.3	53.7±8.50	39.8±12.2	
AA							
PGD_2	163±15.6^{ab}	45.9±6.30^{c}	61.1±6.50^{c}	236±56.6^{a}	124±5.90^{b}	61.3±6.30^{c}	Int
PGE_2	251±19.2^{b}	59.2±4.60^{d}	73.5±7.50^{d}	360±42.5^{a}	176±10.5^{c}	70.2±12.3^{d}	Int
11β-PGE_2	704±51.2^{ab}	150±15.8^{c}	176±26.9^{c}	1009±145^{a}	498±26.3^{b}	192±32.6^{c}	Int
PGF_{2a}	196±11.1^{b}	74.9±6.30^{b}	85.5±11.6^{c}	298±36.2^{a}	160±10.1^{b}	84.3±8.10^{c}	Int
6-keto-$PGF_{1\alpha}$	724±90.9^{a}	257±45.6^{bc}	185±12.9^{c}	820±66.2^{a}	725±64.5^{a}	384±47.0^{b}	Int
TxB_2	53.1±7.70^{A}	28.4±6.90^{B}	19.6±4.30^{C}	106±7.30	58.9±4.50	23.0±4.20	S O
5-HETE	1124±331^{A}	332±108^{B}	695±126^{B}	1720±237	561±196	281±135	O
5-oxo-ETE	314±145^{a}	130±39.7^{c}	252±46.6^{ab}	302±47.4^{a}	98.7±33.3^{c}	152±55.2^{bc}	Int
8-HETE	362±53.0^{A}	153±39.7^{B}	177±31.2^{B}	430±95.8	188±44.3	118±29.0	O
9-HETE	519±116^{A}	190±51.5^{B}	318±38.5^{B}	626±128	205±39.9	206±89.5	O
11-HETE	332±29.1^{b}	137±15.8^{c}	175±21.2^{c}	485±53.8^{a}	278±18.3^{b}	142±19.9^{c}	Int
12-HETE	1198±310	380±91.7	703±208	1219±420	456±113	378±115	
15-HETE	620±115^{b}	170±22.1^{d}	419±57.9^{bc}	1096±47.8^{a}	469±105^{bc}	216±98.5^{cd}	Int
14,15 DiHETrE	30.0±4.30^{A}	9.00±1.30^{B}	19.5±2.10^{B}	31.3±1.40	12.1±2.00	10.3±1.40	O
5,6 DiHETrE	66.9±21.0^{A}	24.3±8.40^{B}	40.6±7.10^{B}	81.5±4.20	28.4±8.40	23.2±5.80	O
11,12 DiHETrE	26.5±5.60^{A}	6.80±1.00^{B}	16.6±2.20^{B}	32.1±3.10	13.3±2.30	8.70±1.20	O
16-HETE	70.7±10.0^{ab}	37.8±9.70^{c}	44.2±9.50^{bc}	80.4±14.1^{a}	37.5±8.70^{c}	42.8±14.9^{bc}	Int
18-HETE	86.5±19.3^{A}	53.4±15.7^{B}	22.3±6.50^{B}	56.9±8.10	27.2±7.20	35.6±18.0	O
n-3 oxylipins							
ALA							
9-HOTrE	299±94.3^{B}	1123±358^{A}	122±22.2^{C}	222±93.5	503±149	28.1±5.30	S O
13-HOTrE	13392±2827^{B}	55429±18729^{A}	6040±856^{B}	10585±4079	29927±7141	6072±2828	O
EPA							

Δ17-6-keto-$PGF_{1\alpha}$	4.10±0.80^{B}	12.2±4.10^{A}	18.6±3.60^{A}	4.40±2.70	22.9±4.90	25.8±4.70		O
5-HEPE	215±66.0^{B}	669±205^{A}	1625±235^{A}	222±53.0	816±286	813±279		O
8-HEPE	48.7±12.7^{B}	145±28.7^{A}	194±20.9^{A}	43.6±25.1	133±24.2	93.9±14.6		O
9-HEPE	157±33.9^{cd}	546±83.4^{b}	1238±137^{a}	76.1±6.70^{d}	424±82.4^{bc}	538±132^{b}	Int	
11-HEPE	71.7±9.40^{C}	272±43.1^{B}	547±80.0^{A}	66.7±11.6	248±16.7	365±50.5		O
12-HEPE	503±168^{B}	1887±403^{A}	1932±209^{A}	244±70.3	959±215	1630±366	S	O
18-HEPE	798±277^{C}	3797±999^{B}	11789±1533^{A}	640±202	4056±866	6493±2022		O
DHA								
4-HDoHE	797±283^{b}	484±150^{b}	1520±259^{a}	819±96.4^{b}	478±159^{b}	466±260^{b}	Int	
7-HDoHE	120±36.2^{B}	86.4±20.0^{B}	241±32.2^{A}	83.8±15.7	70.7±15.9	107±36.1	S	O
8-HDoHE	600±200^{b}	415±114^{b}	1306±221^{a}	456±83.1^{b}	386±125^{b}	404±201^{b}	Int	
10-HDoHE	60.8±13.3^{b}	44.6±8.50^{b}	138±23.7^{a}	50.7±3.40^{b}	50.3±14.0^{b}	46.7±18.0^{b}	Int	
11-HDoHE	144±46.3	96.6±27.3	372±75.7	122±6.10	106±35.0	101±48.5		
13-HDoHE	81.9±18.8^{b}	66.0±12.7^{b}	194±20.7^{a}	76.3±5.80^{b}	68.1±10.3^{b}	65.3±15.7^{b}	Int	
14-HDoHE	397±89.3^{B}	378±75.0^{B}	849±117^{A}	329±48.4	282±55.2	370±74.6	S	O
16-HDoHE	582±142^{b}	429±97.3^{b}	1265±183^{a}	463±44.9^{b}	373±102^{b}	450±164^{b}	Int	
17-HDoHE	840±204^{AB}	499±110^{B}	1606±276^{A}	979±70.7	796±217	1027±302		O
17-keto-DHA	1038±341	570±119	1674±348	845±81.2	776±267	792±310		
19,20 DiHDPE	376±62.0^{AB}	302±58.7^{B}	368±65.3^{A}	94.7±3.90	71.2±9.10	160±21.2	S	O
20-HDoHE	19588±7326	12535±3284	38313±6256	18172±2983	12412±4239	16078±9162		
Non-Enzymatic AA oxylipins								
5-iso PGF2aVI	26.6±2.80^{A}	9.20±2.60^{C}	19.5±3.40^{B}	38.9±4.50	5.70±2.70	23.1±3.80		O

All values represent mean ± SEM (n=4-7). S, sex effect; O, oil effect, Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in male) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

Table 7.9: Dietary oil and protein effects on renal oxylipins in PCK rats (pg/mg dry tissue)

Oxylipin	Casein			Soy protein			
	Soy oil	Flax oil	Fish oil	Soy oil	Flax oil	Fish oil	Effects
n-6 oxylipins							
LA							
9-HODE	13513±1377	6077±1152	4005±491	16128±1227 ^{A}	10241±1100 ^{B}	4893±650 ^{B}	P O
9-oxo-ODE	7344±524	4400±881	3675±745	7594±1169.2^{A}	7115±847 ^{B}	3897±517 ^{C}	O
13-HODE	15001 ±1249 ^{a}	7812±1347 ^{b}	5627±724 ^{b}	18878±1369 ^{a}	14167±1190 ^{a}	5899±760 ^{b}	Int
13-oxo-ODE	3827±314.2 ^{ab}	2782±556 ^{bc}	1997±397 ^{c}	3347±357 ^{abc}	4986±379 ^{a}	2001±279 ^{c}	Int
9,12,13 TriHOME	1833±318	1368±296	836±112	1723±250 ^{A}	1994±300 ^{A}	1063±76.3 ^{B}	O
9,10,13 TriHOME	4740±763	3800±520	2442±306	5414±691 ^{A}	6260±710 ^{A}	3356±331 ^{B}	P O
9,10-DiHOME	142±21.3	184±31.7	54.7±5.10	276±16.3 ^{A}	301±24.9 ^{A}	113±16.1 ^{B}	P O
12,13-DiHOME	31.8±4.10	29.2±7.50	15.3±1.60	79.9±8.60 ^{A}	80.7±9.10 ^{A}	55.3±7.50 ^{B}	P O
GLA							
13-HOTrE-γ	367±62.5	140±31.9	101±8.90	335±57.1 ^{A}	204±32.6 ^{B}	104±21.0 ^{C}	O
DGLA							
8-HETrE	481±22.6	220±50.0	277±28.1	503±47.5 ^{A}	284±24.5 ^{B}	251±35.6 ^{B}	O
15-HETrE	325±22.7	145±27.9	164±18.3	322±48.3 ^{A}	230±23.0 ^{B}	187±29.4 ^{B}	O
AA							
PGD_2	283±33.7	166±40.1	228±50.1	275±19.7^{A}	154±23.4^{B}	152±32.8^{B}	O
PGE_2	249±12.3 ^{a}	115±18.4 ^{c}	89.3±9.00 ^{c}	194±16.6 ^{ab}	141±17.9 ^{bc}	96.2±6.50 ^{c}	Int
11 β-PGE_2	572±30.2 ^{a}	231±36.2 ^{b}	200±19.7 ^{b}	468±28.4 ^{a}	289±36.8 ^{b}	214±20.1 ^{b}	Int
PGF2a	382±29.0	167±28.3	135±10.7	330±23.3 ^{A}	165±20.6 ^{B}	114±9.60 ^{B}	O
6-keto-$PGF_{1\alpha}$	366±23.6 ^{a}	166±18.0 ^{dc}	131±15.1 ^{d}	287±23.5 ^{ab}	271±33.4 ^{bc}	151±13.6 ^{d}	Int
TxB_2	289±33.8	149±21.5	113±23.4	200±20.4 ^{A}	162±19.7 ^{B}	87.5±8.30 ^{C}	O
5-HETE	4719±352	1916±424	1761±186	4145±341 ^{A}	2392±217 ^{B}	1858±103 ^{B}	O
5-oxo-ETE	613±61.1	297±69.2	285±60.2	525±109 ^{A}	440±85.1 ^{B}	265±34.8 ^{B}	O
8-HETE	999±44.8	477±95.4	382±70.6	822±139 ^{A}	497±53.6 ^{B}	374±52.7 ^{B}	O
9-HETE	3121±188	1098±271	1106±176	2866±192 ^{A}	1317±139 ^{B}	1100±115 ^{B}	O
11-HETE	3884±270	1754±320	1641±263	3266±268 ^{A}	2220±295 ^{B}	1384±180 ^{B}	O
12-HETE	1881±128	565±117	645±107	1524±195 ^{A}	794±111 ^{B}	624±72.8 ^{B}	O
15-HETE	6269±395	2278±511	2295±209	5524±460 ^{A}	3155±366 ^{B}	2055±260 ^{B}	O
15-oxo-ETE	2591±217	1886±288	1348±302	2166±502 ^{A}	1723±283 ^{AB}	1535±249 ^{B}	O
5,6-DiHETrE	50.5±8.30	28.5±6.30	23.3±5.60	59.9±15.8 ^{A}	51.9±10.4 ^{AB}	24.4±2.60 ^{B}	O
8,9-DiHETrE	40.8±3.20	26.6±5.30	15.7±1.80	43.1±4.70 ^{A}	31.6±3.30 ^{B}	15.4±2.40 ^{C}	O
11,12-DiHETrE	74.2±10.5	83.3±14.9	34.8±5.80	88.6±4.40 ^{A}	87.9±15.8 ^{A}	25.7±4.50 ^{B}	O

14,15-DiHETrE	55.8±3.60	38.9±8.30	26.2±3.80	64.3±10.4 ^{A}	42.7±5.80 ^{B}	27.8±3.70 ^{C}	O
16-HETE	285±23.4	143±31.9	129±11.2	261±37.5 ^{A}	195±33.5 ^{B}	102±16.1 ^{B}	O
n-3 oxylipins							
ALA							
9-HOTrE	870±113 ^{bc}	2314±613 ^{b}	307±56.6 ^{d}	861±117 ^{c}	5548±497 ^{a}	305±48.1 ^{d}	Int
9-oxo-OTrE	325±76.4 ^{cb}	806±237 ^{b}	179.6±30.7 ^{cd}	330±60.0 ^{cb}	1683±231 ^{a}	107±20.1 ^{d}	Int
13-HOTrE	90212±11000	289120±101067	43982±7719	117202±17767 ^{B}	534127±102839 ^{A}	35797±5057 ^{C}	O
EPA							
5-HEPE	1573±190	2280±362	6282±284	1649±422 ^{C}	2837±360 ^{B}	5756±705 ^{A}	O
9-HEPE	416±56.5	2089±495	4713±378	342±60.8 ^{C}	1829±357 ^{B}	3998±547 ^{A}	O
11-HEPE	166±20.9	784±58.3	1717±52.6	154±22.4 ^{C}	832±73.3 ^{B}	1493±92.5 ^{A}	O
12-HEPE	205±14.9 ^{d}	686±103 ^{c}	2339±167 ^{a}	172±11.5 ^{d}	953±33.4 ^{b}	2049±134 ^{a}	Int
15-HEPE	823±111	1688±295	5428±402	531±91.9 ^{C}	2007±173 ^{B}	4186±634 ^{A}	O
18-HEPE	1431±148	5169±878	20424±3060	1545±298 ^{C}	6635±607 ^{B}	18858±1543 ^{A}	O
DHA							
4-HDoHE	880±73.3	561±89.7	2686±346	788±117 ^{B}	841±95.4 ^{B}	2792±172 ^{A}	O
7-HDoHE	124±15.8	127±35.6	383±39.0	138±10.7 ^{B}	145±29.7 ^{B}	308±43.1 ^{A}	O
10-HDoHE	114±8.90	100±21.1	407±63.1	122±18.7 ^{B}	109±17.1 ^{B}	270±37.6 ^{A}	O
11-HDoHE	376±31.3	351±67.6	1130±150	305±25.3 ^{B}	352±52.8 ^{B}	1039±88.7 ^{A}	O
13-HDoHE	94.5±8.70	74.1±8.70	290±37.4	88.8±11.0 ^{B}	115±24.4 ^{B}	220±27.3 ^{A}	O
14-HDoHE	499±42.6 ^{b}	306±40.9 ^{c}	1610±207 ^{a}	480±39.0 ^{b}	428±28.8 ^{bc}	1279±111 ^{a}	Int
16-HDoHE	342±29.7	245±41.5	1141±132	349±51.7 ^{B}	328±24.6 ^{B}	945±66.6 ^{A}	O
17-HDoHE	1520±122	1241±237	4861±407	1423±185 ^{B}	1479±100 ^{B}	4597±349 ^{A}	O
19,20 DiHDPE	47.8±5.50	45.2±6.30	126±12.2	45.4±6.40 ^{B}	63.7±9.40 ^{B}	148±19.4 ^{A}	O
20-HDoHE	6740±371	4018±646	22501±2987	7381±611 ^{B}	5936±332 ^{C}	22221±1602 ^{A}	P O
Non-enzymatic AA oxylipins							
5-isoPGF$_{2\alpha}$VI	259±20.3	105±26.3	84.0±13.5	204±32.4 ^{A}	120±13.9 ^{B}	85.2±10.1 ^{B}	O
8-iso-PGF$_{2\alpha}$	1088±61.2 ^{a}	392±56.4 ^{b}	286±22.6 ^{c}	906±69.3 ^{a}	554±69.8 ^{b}	294±28.6 ^{c}	Int

All values represent mean ± SEM (n=5-8). P, protein effect; O, oil effect, Int, interaction or Wilcoxin's test used for non-normal data. Differing uppercase superscripts (shown in soy protein groups) indicate differences in oil effects. Differing lowercase superscripts indicate differences in simple effects.

7.4.2 Effect of dietary lipids on renal n-3 PUFA derived oxylipins

In contrast to the similar patterns in n-6 fatty acid derived oxylipins in response to dietary oil type, those derived from n-3 fatty acids had differing patterns. Oxylipins derived from ALA were generally higher with flax oil feeding, while those derived from EPA or DHA were highest in the fish oil groups. Total ALA oxylipins were 2-5.5 times higher in the flax compared to the soy oil groups in all four studies, although not always significantly in all subgroups. On the other hand, fish compared to soy oil consumption did not increase ALA oxylipins, but resulted in 18-79% lower ALA oxylipins, but this was only significant in PCK rats. Compared to flax, the fish oil groups had 85-90% lower total ALA oxylipins across the studies, with most of the differences being significant (Figure 7.1, Tables 7.2-7.5). These patterns generally reflected the differences in the 2-3 individual ALA oxylipins present in each study (Tables 7.6-7.9).

Total EPA oxylipins were 4.5 to 9.1 times higher with flax compared to soy oil consumption in the mouse models, and 2.5-3.3 times higher in the PCK rats, although this latter difference was not significant. On the other hand, fish compared to soy oil consumption resulted in 8 to 16 times higher total EPA oxylipins across all four studies. Fish compared to flax oil differences were not as great, but total EPA oxylipins were 1.8 to 2.8 times higher in Pkd1 (5wk) mice, Pkd2 mice, the male subgroup of Pkd1 (1wk) mice and PCK rats (Tables 7.2-7.5). Of the 6-8 individual EPA oxylipins quantitated in each study, 100% were higher in both flax and fish compared to soy oil groups in all four studies, while fish compared to flax oil groups had higher levels in 38-100% of individual EPA oxylipins across studies (Tables 7.6-7.9).

Total DHA oxylipins were not affected by flax compared to soy oil consumption in any of these studies. On the other hand, fish compared to soy oil consumption resulted in 1.8 to 3 times higher total DHA oxylipins in both Pkd1 models and in PCK rats. Fish compared to flax

oil consumption also resulted in 1.5 to 4 times higher DHA oxylipins in Pkd1 (1wk) mice, soy protein subgroup of Pkd1 (5wk) mice and in the PCK rats. No oil effects on total DHA oxylipins were observed in Pkd2 mice (Tables 7.2-7.5). 10-14 individual DHA oxylipins were quantitated in each study. Flax compared to soy oil consumption only increased 14% of DHA oxylipins in Pkd1 (1wk) mice, but none in the other studies. However, fish compared to soy oil consumption resulted in 100% of these individual oxylipins being higher in Pkd1 (1wk) mice and PCK rats, while in Pkd1 (5wk) and Pkd2 mice, 50% and 25%, respectively, of these individual oxylipins were higher overall, with an additional 33 to 44% being higher in one of the subgroups. Fish compared to flax oil consumption resulted in higher levels in 42-100% of individual DHA oxylipins across all four studies.

7.4.3 Effect of dietary protein on renal oxylipins

Two sources of protein were provided to the Pkd1 (5wk) mice and PCK rats. Effects of soy protein compared to casein on renal oxylipins were observed, but only consistently in those derived from LA. Total LA oxylipins were 1.2 and 1.3 times higher in soy protein compared to casein fed Pkd1 (5wk) mice and PCK rats, respectively (Figure 7.2, Tables 7.2 & 7.5). Among 8 individual LA oxylipins quantified, 50% were higher in the soy protein groups in both studies. Among these, 9,10-dihydroxy octadecenoic acid (DiHOME) and 12,13-DiHOME were always higher with soy protein consumption in both studies (Tables 7.6 & 7.9). There was no effect of dietary protein on total DGLA or AA oxylipins in either study.

The effect of dietary protein on n-3 PUFA derived oxylipins also was minimal. In Pkd1 (5wk) mice, total EPA oxylipin levels were not affected by soy protein feeding, while ALA oxylipins were 1.5 times higher only in the male flax oil subgroup and DHA oxylipins were 1.9

times higher in only in the male fish oil subgroup given soy protein. However, no individual n-3 fatty acid derived oxylipins were affected by protein source in these mice (Tables 7.2 & 7.6). In PCK rats, total ALA, EPA, and DHA derived oxylipins were not affected by dietary protein, and only 1 of 19 n-3 fatty acid derived oxylipins were higher with soy protein feeding, with an additional 3 being higher in the flax oil subgroups (Tables 7.5 & 7.9).

7.4.4 Effect of sex on renal oxylipins

The 3 mouse studies included both males and females. In both Pkd1 mouse models, total AA derived oxylipins were 1.3 times higher in female compared to male kidneys (Figure 7.3, Tables 7.2 & 7.3). Of the 12 AA oxylipins quantitated in each of the Pkd1 studies, 59% and 29% were higher in female Pkd1 (5wk) and Pkd1 (1wk) mice, respectively. An additional 35% of these AA oxylipins were higher in females in soy oil subgroups of Pkd1 (1wk) mice (Tables 7.6&7.7). In Pkd2 mice, although total AA oxylipins were not affected by sex, 6% of individual AA oxylipins were higher in females in all subgroups, and an additional 41% of AA oxylipins were higher in females in some subgroups (Table 7.8).

In contrast to AA oxylipins, other n-6 PUFA derived oxylipins that were affected by sex were higher in male kidneys. Total oxylipins formed from LA, GLA and DGLA were not affected by sex in any of the 3 studies (Tables 7.2-7.4), but 12-50% of individual LA oxylipins were higher in males across all 3 studies (Tables 7.6-7.8).

The effect of sex on n-3 PUFA derived oxylipins also was minimal and opposite to its effect on AA oxylipins. Among the totals of n-3 PUFA derived oxylipins, only total DHA oxylipins were (1.3 times) higher in males, but only in Pkd1 (1wk) mice (Table 7.7). Among the 3 individual ALA oxylipins quantitated in each study, only 1 was higher in males in each of the

Pkd1 (5wk) mice, Pkd2 mice, and the flax oil subgroup of the Pkd1 (1wk) mice. With respect to EPA oxylipins, 2-4 out of 7 were higher in males across all three studies. Among 12-14 individual DHA oxylipins, 17-57% of them were higher in males overall, with an additional 0-42% being higher in males in some subgroups (Tables 7.6-7.8).

7.5 Discussion

In all four studies, n-6 PUFA derived renal oxylipins were lower in animals provided flax or fish compared to soy oil, but the levels were generally much lower in the animals with fish oil diets. Reduction of n-6 PUFA derived oxylipins in response to fish oil consumption has been observed previously in rodent kidneys, but the number of oxylipins examined was limited (10, 616). Similar effects of fish oil on a larger range of plasma n-6 oxylipins have been reported in humans (468, 617). Flax oil also is effective in lowering n-6 PUFA derived oxylipins in rodent kidneys and human plasma (121, 514). The current study extends these findings to a larger profile of renal oxylipins in a direct comparison of fish and flax oil in the same model. The greater effect of fish compared to flax oil occurred despite the fact that the EPA and DHA content of the fish oil diet was less than half that of the ALA content of the flax oil diet, indicating that EPA and DHA have a greater effect than ALA on the n-6 PUFA derived oxylipins.

In contrast to the reduction in the n-6 derived oxylipins, the n-3 derived oxylipins were increased with both flax and fish oil feeding, but the effect on ALA oxylipins was greater with flax oil, while the effect on EPA and DHA oxylipins was greater with fish oil. The elevation of ALA oxylipins with flax oil feeding is consistent with flax oil studies in rodent renal tissues and in plasma from human feeding studies (121, 505, 620). Flax oil feeding also increased EPA

oxylipins, which can be attributed to the conversion of ALA to EPA in the tissue (621, 622). This has been observed in rodent kidney and human plasma with flax oil feeding (121, 514). However, fish oil feeding in the current study resulted in much higher levels of EPA derived oxylipins, even though the EPA level in the fish oil diet was present at a level one-fourth that of the ALA in the flax oil diet. Thus, directly supplying EPA in the diet is more effective in elevating EPA oxylipins than providing much higher levels of ALA. Another difference in the effect of fish vs flax oil on renal oxylipins was that while fish oil resulted in higher DHA oxylipins, flax oil did not. Dietary flax oil also did not elevate renal DHA oxylipins in a previous study in obese rats (121). In contrast, flax oil feeding increased the levels of 10 DHA derived oxylipins in a mouse model of cystic kidney disease in which DHA oxylipins were reduced with disease (8). Interestingly, 4-week consumption of flaxseed in humans decreased the levels of DHA oxylipins (514). On the other hand, consumption of fish oil raises the plasma levels of DHA derived oxylipins in healthy human subjects (467, 468). In the four studies presented herein where fish and flax oil were directly compared, fish oil containing DHA elevated renal DHA oxylipins while flax oil did not. Conversely, the fish oil diet did not increase renal ALA oxylipins, suggesting that retro-conversion of EPA and DHA to ALA does not occur to a significant extent in the kidney with these diets. This is consistent with the effect of fish oil on tissue ALA levels (623-626).

The differential effects of flax and fish oil on n-3 PUFA derived oxylipins may help explain their differences in effects on kidney health. In some kidney diseases flax oil has beneficial effects (6, 505, 627-630), while fish oil has been shown to have beneficial effects (543, 631-633), no effect (16, 634-636) or in some cases detrimental effects (9, 637). In one study that investigated the effects of flax oil and algal oil rich in DHA on renal disease in the same model,

flax oil slowed disease progression, while the DHA oil enhanced disease progression(9). While many studies have shown that oxylipins derived from EPA and DHA have anti-inflammatory and pro-resolving properties (20, 461), much less is known about ALA oxylipins. One study demonstrated that ALA derived HOTrEs have anti-inflammatory properties in-vitro (515) and another showed that 9,16-DiHOTrE inhibits human platelet aggregation (147). Thus it is possible that distinct effects of flax vs fish oil may be due to the different oxylipin profiles that result from fish vs flax oil feeding.

There is very limited literature on the effect of dietary protein on the synthesis of oxylipins. In the two studies with different dietary protein sources, LA derived oxylipins were elevated with soy protein feeding, while oxylipins from other PUFA generally were not affected. Oxylipin changes in response to dietary soy protein previously have been observed, but these changes coincided with effects on disease, so whether these effects were secondary to disease effects could not be ascertained (10, 521). In the current studies, dietary soy protein had minor (PCK rat) or no [Pkd1 (5wk) mouse] effect on disease, suggesting that the effect of dietary soy protein on oxylipins was independent of disease effects. Tissue LA levels have been shown to increase with soy protein consumption in renal and hepatic tissues of rats with renal disease (638) and in liver and lymphocytes of normal rats (639). This increase in LA levels has been attributed to the ability of soy protein to inhibit the activity of the Δ6-desaturase mediated conversion of LA to longer chain n-6 PUFA (521, 522). This may help explain the increased formation of LA oxylipins in our studies. However, there is no evidence that this putative Δ6-desaturase inhibition reduced the levels of the oxylipins derived from the downstream PUFA, possibly because the fatty acid pool was not depleted sufficiently to affect oxylipin levels. Further studies examining

tissue Δ6-desaturase and FA levels are warranted to further understand this novel effect of soy protein on LA derived oxylipins.

Implications of elevated levels of LA oxylipins, and the DiHOME in particular, on renal health are not well understood. However, it has been shown that epoxy-octadecenoic acids (EpOME) and DiHOMEs derived from LA have cytotoxic effects in renal proximal tubular cells (319, 640) and DiHOMEs have been reported to induce chemotaxis in human neutrophils (641). Effects of other LA oxylipins have been demonstrated in other tissues as well, including anti-inflammatory and anti-proliferative properties in the skin (337), inhibition of tumor cell adhesion (242) and reduction in leukotriene B_4 secretion from leukocytes (642). In humans, LA oxylipins, HODE and oxoODEs have been implicated in oxidative stress and inflammation (427). The role of LA oxylipins in renal physiology largely remains to be elucidated.

In the current studies in which sex effects were examined, only AA derived oxylipins were higher in females compared to males. Studies investigating the effect of sex on the oxylipin profile are rare and have mostly examined a limited number of AA oxylipins. For example, plasma PGE_2 and prostacyclin levels are higher in female than in male rats (534) and female rats and mice have enhanced urinary excretion of PGE_2, and TxB_2 (531, 533). These sex differences may be due to differences in formation and/or degradation of oxylipins, as female rats and mice have greater expression of COX-2 (531, 605) and PGE_2 synthase (531), and estradiol suppresses the activity of 15-hydroxyPG dehydrogenase, a PG-degrading enzyme in rat renal tissue (532, 535), and in human fetal tissue (643). On the other hand, females also have a higher activity of prostaglandin 9-ketoreductase in rat renal tissues (536), suggesting that other factors also are involved. For example, tissue FA levels may be different in males and females, as suggested in

human studies that indicate that females may have higher levels of plasma AA compared to males (602-604). Another potential factor affecting sex differences in renal oxylipin levels could be the role of transporters associated with clearance of oxylipins. Hatano et al recently reported that the expression of a transporter responsible for clearance of PGE_2 was lower, and PGE_2 concentrations were higher in renal cortex of female compared to male rats (537). Thus, differential activity of enzymes involved in AA metabolism, higher levels of tissue AA and lower expression of transporters may have contributed to the higher levels of AA derived oxylipins in females in our studies.

It is notable that the effect of sex on AA derived oxylipins is unique and different from the effects on oxylipins from other n-6 and n-3 PUFA, which were either higher or not different in males compared to females. This is of particular importance because conclusions on oxylipins have almost exclusively been based on the literature available on the oxylipins produced from AA. The current study indicates that findings on sex differences in AA derived oxylipins may not be generalizable to other PUFA.

The diet-induced changes on renal oxylipins in these studies are unlikely to be due to their effects on disease. As published elsewhere, diet had minimal effects on disease in the PCK rats and Pkd2 mice used in this study (618), and in both of the Pkd1 mouse studies disease progression was very minor, with no effect of diet or sex on disease progression. Thus, consistent sex and diet effects on oxylipins across varying degrees of disease in multiple studies herein suggest that the results are likely to be independent of disease. Nevertheless, whether these effects are present in normal models remains to be demonstrated.

In conclusion, these studies provide novel data directly comparing flax and fish oil on the renal oxylipin profile. Fish compared to flax oil resulted in greater reduction in n-6 PUFA

derived oxylipins and greater increase in EPA and DHA derived oxylipins, whereas flax oil was more effective in elevating ALA derived oxylipins. Soy protein resulted in higher levels of oxylipins from LA, while females compared to males displayed higher levels of AA oxylipins. Further studies are required to elucidate how these novel effects of diet and sex on the renal oxylipin profile affect renal health and disease.

Chapter 8

8. General discussion and conclusions

8.1 Introduction

Four studies presented herein examined the effects of dietary flax oil, fish oil, and soy protein and sex on the disease progression and oxylipin levels in orthologous models of PKD. Dietary interventions and sex had very few effects on disease. A detailed profile of over 50 oxylipins in the renal tissue was established for the first time in mouse and rat models in normal and diseased animals. Comparison of normal to diseased animals revealed distinct and consistent disease specific alterations in oxylipins. Additionally, this is the first study comparing flax and fish oil side by side in the same model for its effects on disease and oxylipin production. Dietary interventions with these oils generated distinct effects on n-3 and n-6 PUFA derived renal oxylipins. This is also the first study to report unique effects of dietary soy protein and sex on oxylipins derived from LA and AA, respectively.

8.2 Dietary interventions and disease modification in orthologous models

Rationale for investigating the effects of flax oil, fish oil and soy protein in the disease stems from previously reported benefits of these dietary interventions in Han:SPRD-*Cy* rats and *pcy* mice which are non-orthologous models of human PKD (6, 10, 509, 510). Our experiments included 3 orthologous models of which 2 were used for the first time in a dietary intervention study. Overall, dietary interventions with oils enriched in n-3 PUFA early in the development of PKD displayed no benefits but caused some minor detrimental effects on disease in orthologous models of PKD. In the Pkd1 mouse studies, disease progression was lesser and variable compared to the other two models and dietary interventions had minimal effect, except that fish

oil tended to increase kidney size. In Pkd2 mice while most parameters were not affected, kidney weight was slightly higher with flax and fish oil compared to soy oil. In PCK rats, while there was no effect of dietary oil on most of the markers measured, both flax and fish oil resulted in larger kidneys with higher water content, greater kidney cyst and lumen area and elevated creatinine clearance compared to soy oil. Similarly, dietary soy protein also increased kidney weight and creatinine clearance compared to casein. Lack of benefit in these orthologous animals is similar to previous findings in female PCK rats (16), which showed no benefit of either fish oil or soy protein. Lack of benefit or detrimental effects of fish oil has been reported also in non-orthologous models (9, 517, 543). However, flax oil related findings from our study are in sharp contrast to non-orthologous models where consistent benefits were observed with flax oil (8, 9, 509). This indicates that the benefits/effects observed in non-orthologous models may not be directly applicable to human disease. Therefore, our study demonstrates that there is no evidence from orthologous models to support dietary recommendation of n-3 PUFA or soy protein to human PKD patients. As a result of these findings, the US PKD foundations removed its recommendations for increasing dietary soy protein and n-3 PUFA containing oils after our manuscript (chapter 4) was published (13).

8.3 Disease specific alterations in renal oxylipins

Different models used in the study displayed varying progression of disease. None the less, there were consistent distinctions between normal and diseased animals in all three models. For example, total COX oxylipins were higher in diseased animals in all three models. Elevation in COX derived oxylipins in these models is similar to previously reported COX oxylipin alterations in non-orthologous Han:SPRD-*Cy* rat (10, 485, 513, 585) and *pcy* mouse (8, 18)

models. This indicates that COX inhibition thorough dietary/pharmacological intervention may help slow down the disease progression in orthologous models.

Interestingly, from more than 50 oxylipins quantitated, there were several individual oxylipins from AA, generated via COX pathway, which were consistently higher in disease. 6-keto-$PGF_{1\alpha}$, was higher with disease in Pkd 1 females, Pkd 2 mice and PCK rats, PGE_2 levels were higher Pkd1 mice and PCK rats, and PGD_2 was higher in both mouse models. A higher level of 6-keto-$PGF_{1\alpha}$ is reported in non-orthologous models (8, 10, 18) as well as in acute kidney injury patients (644). 6-keto-$PGF_{1\alpha}$ is a stable metabolite of PGI_2. PGI_2, PGD_2 and PGE_2 cause elevation in cAMP levels (488, 571, 579, 645), which in turn stimulates epithelial cell proliferation and fluid secretion (572), thus accelerating the disease progression. Additionally, PGE_2 is an important mediator of renal blood flow, vascular tone, oxidative stress, inflammation, fibrosis and apoptosis in renal cells (646-650). It is clear from both orthologous and non-orthologous models that these oxylipins play very crucial roles in accelerating renal cystic disease. Therefore, studies into interventions targeting these molecules are warranted.

In contrast to increased production of renal COX oxylipins, changes in LOX and CYP oxylipins in diseased kidneys were not consistent across studies. Although there were some individual oxylipins that differed between normal and diseased animals, total LOX and CYP oxylipins largely remained unaffected in ADPKD models, whereas in PCK rats CYP-epoxygenase derived oxylipins were lower with disease. This was contrary to the results from non-orthologous models where lower levels of LOX products are reported (8, 10, 18, 585). This discrepancy might be due the fact that animals in our studies were in the earlier stages of disease whereas the most studies in non-orthologous models were in advanced stages of disease. It also

indicates that mechanisms of disease progression may be different in models of NPHP compared to models of PKD. *pcy* mouse is a model of NPHP and Han:SPRD-*Cy* rats have a mis-formed Anks6 protein which form complexes with NPHP proteins. Distinct patterns of differences in oxylipin in ADPKD and ARPKD models indicate that the mechanisms of disease progression are different in these two conditions as well. Whether these modifications in oxylipin levels are causative or are the result of disease remains to be further investigated. However, elevation in COX derived oxylipins in Pkd1 mice with very little disease suggests that oxylipin alterations are initiated much earlier than the manifestation of disease outcomes.

8.4 Effect of dietary oils on renal oxylipins

Renal oxylipin analysis revealed distinct effects of soy, flax and fish oil on oxylipins produced from both n-6 and n-3 PUFA. Total and several individual LA and AA derived oxylipins were lowered by both flax and fish oil compared to soy oil, but fish oil had a much greater lowering effect. Other n-6 PUFA derived oxylipins followed the same pattern with fish oil having the lowest levels, and flax oil with intermediate levels. Interestingly, a similar pattern was not observed in the n-3 derived oxylipins across PUFA. ALA derived oxylipins were highest in the flax oil groups and EPA and DHA derived oxylipins were highest in the fish oil groups. Flax oil feeding did not affect DHA derived oxylipins and fish oil feeding did not affect ALA derived oxylipins.

Reduction in a limited number of n-6 PUFA derived oxylipins in response to fish oil consumption has been observed previously in rodent kidneys (10, 616)and in humans (468, 617). Flax oil also is effective in lowering n-6 PUFA derived oxylipins in rodent kidneys and human plasma (121, 514). The elevation of ALA and EPA derived oxylipins with flax oil feeding is

consistent with flax oil studies in rodent renal tissues and in plasma from human feeding studies (121, 505, 620). However, fish oil was more effective in elevating renal EPA oxylipins. Flax and fish oil were also different from each other in the effects on DHA oxylipin levels in that fish oil resulted in higher DHA oxylipins, but flax oil did not. Similar to our results, consumption of fish oil elevates the plasma levels of DHA oxylipins in healthy human subjects. Again, consistent with our observations, dietary flax oil did not elevate renal DHA oxylipins in obese rats (121), and lowered DHA oxylipins in humans (514). However, in a mouse model of cystic kidney disease in which DHA oxylipins were reduced with disease, flax oil feeding increased the levels of several DHA derived oxylipins (8). While previous studies investigated the effect of flax and fish oil individually on a limited number of oxylipins, our study provides a side-by-side comparison of efficacy of these dietary oils in reducing n-6 oxylipins and elevating n-3 oxylipins in a larger profile. It is worth noting that the dietary oil induced differences in oxylipins did not parallel the alterations in disease in these animals. Since our observations were made on animals with very early stage disease, the results may be generalizable to normal animals.

8.5 Effect of dietary soy protein on renal oxylipins

This is the first study to investigate the effect of dietary protein on a large number of oxylipins. Dietary soy protein had a unique effect on elevating LA derived oxylipins, whereas oxylipins from other n-6 and n-3 PUFA remained largely unaffected. Since dietary protein had very little effect on disease in these animals, the effect of dietary soy protein on oxylipins was likely independent of disease effects. Soy protein inhibits the activity of Δ6-desaturase that converts LA to longer chain n-6 PUFA (521, 522). Consequently, in normal and diseased rats,

soy protein feeding is reported to elevate tissue LA levels (638, 639). Such an increase in tissue LA, acting as substrate for oxylipin production might have caused the increase in LA derived oxylipins in our study. Implications of LA oxylipins in renal disease are less studied. However, LA metabolites possess anti-inflammatory and anti-proliferative properties (337) and causes inhibition of tumour cell adhesion (242) and reduction in leukotriene B_4 secretion (642). In humans, LA oxylipins, HODE and oxoODEs have been implicated in oxidative stress and inflammation (427). EpOMEs and DiHOMEs derived from LA have cytotoxic effects in renal proximal tubular cells (319, 640). DiHOMEs have also been reported to induce chemotaxis in human neutrophils (641). Further studies examining tissue Δ6-desaturase and FA levels and renal effects of LA oxylipins are warranted to understand the consequences of the effect of soy protein on LA derived oxylipins.

8.6 Sex effects on renal oxylipin profile

In the studies where effect of sex was investigated, only AA derived oxylipins were higher in females compared to males. This is a novel finding since studies examining sex effects on oxylipins in kidney, or any other tissue are rare. From studies on limited number of oxylipins, it is known that PGE_2 levels are elevated in females compared to males in plasma of rats (534). Also, female rats and mice have enhanced urinary excretion of PGE_2, and TxB_2 (531, 533). Females have greater expression of enzymes generating AA oxylipins (531, 605), and a lower activity of enzymes that degrade these oxylipins (532, 535, 643). Lower expression of AA oxylipin transporters responsible for AA oxylipins is also reported in renal tissue resulting in accumulation of those oxylipins (537). These factors may have contributed to the higher levels of

AA derived oxylipins in females in our studies. It is important to note that effect of sex on AA oxylipin is unique and different from oxylipins from other n-6 and n-3 PUFA, thus findings on sex differences in AA derived oxylipins may not be generalizable to other PUFA.

8.7 Conclusions

In conclusion, our studies do not support increasing dietary soy protein and oils containing n-3 fatty acids in PKD. These findings have already changed nutritional recommendations for PKD patients in the US. Also, for the first time, a detailed profile of renal oxylipins was established in normal and diseased rodent models. With respect to oxylipin alterations, elevation of COX derived oxylipins with disease was consistent in all orthologous models studied, and is in agreement with previous results from non-orthologous models. This emphasizes the potential for COX inhibition in the treatment of PKD. Our studies also provided novel data directly comparing flax and fish oil on the renal oxylipin profile. Fish oil resulted in greater reduction in n-6 PUFA oxylipins and greater increase in EPA and DHA oxylipins compared to flax oil, whereas flax oil was more effective in elevating ALA derived oxylipins. Dietary soy protein resulted in higher levels of oxylipins from LA, while females compared to males displayed higher levels of AA oxylipins.

Chapter 9

9.1 Future research directions

There were several novel findings needing further exploration revealed in these studies. First, whether the dietary interventions with soy protein and oils containing n-3 PUFA can derive benefits in advanced stages of disease is not clear from our studies. Our studies found no benefits in animals in early stage of disease, but previously reported benefits were seen in animals with advanced disease.

Another important finding is the elevation in COX oxylipin that was consistent across all orthologous models in our studies and non-orthologous models in previous studies. There has been some research on using COX inhibition as a disease modifying strategy. This needs to be further explored to unearth optimal dosage and duration for PKD patients. It is also important to understand the role played by CYP-epoxygenase enzyme derived oxylipins in ARPKD, as this pathway was altered in PCK rats.

For the first time, we have shown that females have higher individual and total AA derived oxylipins. The reasons and consequences of this in health and disease need to be examined in order to establish differential approaches in treatment for female and male patients.

Differences between fish oil and flax oil on the effects of renal oxylipins are demonstrated in our studies. Oxylipins from ALA, EPA and DHA might have distinct effect on renal health. Effects of oxylipins from each individual PUFA needs to be further examined.

Another discovery was the unique effect of soy protein in elevating LA derived oxylipins. LA oxylipins constitute the majority of oxylipins in the renal pool, and the implications of alterations to these oxylipin levels are poorly understood. Exploration in this frontier might offer

mechanistic insight to beneficial role of soy protein in renal disease. Several elements in soy protein have been postulated to influence lipid metabolism. It needs to be further examined whether the effects of soy protein stems from its isoflavon content, amino acid composition, methionine/glycine ratio or bioactive peptides derived from soy protein.

9.2 Strengths and limitations

For the first time, three orthologous models were used to determine if n-3 PUFA containing oils and soy protein are beneficial in PKD. The results concurrently established that there is no evidence to recommend increased n-3 PUFA or soy protein intake. This was also the first study to establish the renal oxylipin profile in normal rats and mice (male and female). Also, a diseased related alteration in COX pathway was revealed in these models. The agreement between models provides further confidence in this, revealing a potential opportunity for medical intervention. Novel effects of dietary protein and sex were also established. A side-by-side comparison of flax and fish oil for its effects on disease and renal oxylipins has also never been undertaken before.

However, the study also had several limitations. Pkd1 mice used in the study were in early stages of disease; this might have potentially reduced the effect of diet on disease. Also, sex effect was not studies in PCK rats. In Pkd2 mice, identification of diseased animals was not possible prior to termination. This resulted in some groups having low sample sizes to allow dietary protein comparison. Another drawback is that the studies did not explore potential mechanism regarding how dietary protein effects renal oxylipin levels.

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APPENDICES

Appendix A: Animal ethics approval

UNIVERSITY OF MANITOBA | Ethics
Office of the Vice-President (Research)

CTC Building
208 - 194 Dafoe Road
Winnipeg, MB R3T 2N2
Fax (204) 269-7173
www.umanitoba.ca/research

30 March 2011

TO: Dr H. Aukema, Department of Nutritional Sciences
R2033-1 SBGHRC, 351 Tache Avenue

FROM: Dr S. Shefchyk, Chair, Bannatyne Campus Protocol Management and Review Committee [redacted]

RE: **"Dietary interventions in animal models of polycystic kidney disease"**

Please be advised that your Animal Care Utilization Protocol, reference **11-007**, has received **approval** by the Bannatyne Campus Protocol Management & Review Committee and is valid until **April 30 2012**. The procedures described by you in the protocol have placed this research in the Category "**D**" of invasiveness.

Regarding the animal ethics requirement for the visiting scientist: Based on the PI's knowledge of the visiting scientist's involvement in the work (i.e. which procedures on animals, degree of involvement of Dr Yamaguchi including length of stay, participation in the design of the experiments and sample size determination), the PMRC suggests that Dr Yamaguchi be given a guest password to the on-line course material related to the CCAC/ethics and that Dr Aukema discuss those aspects of the CCAC and UM regulations that are relevant to Dr Yamaguchi's participation in the proposed work here in Canada. Please email autp@cc.umanitoba.ca.

It is understood that these animals will be used only as described in your protocol. The protocol must be kept current. Should changes become necessary, very minor alterations can be made with the prior written approval of a university Veterinarian and written notification of the Chair of the Bannatyne Campus Protocol Management and Review Committee. More substantive changes will require resubmission to and reassessment by the Bannatyne Campus Protocol Management and Review Committee. If approved, this will result in the assignment of a new protocol reference number.

Failure to follow this protocol, or renew it prior to the expiry date, will result in the termination of your ability to continue using or ordering animals. The protocol reference number must be used when ordering animals.

SS/ck

copy: Dr R. Aitken, Director, RO Burrell Lab
Ms T. Whittington, PAM/Education Technician
Ms D. Borowski, LATC

Appendix B:

Method for urinary protein assay: total protein determination

Modified Bradford assay (Bradford MM, Anal Biochem. 1976 May 7;72:248-54.)

1. Take samples out of the freezer to thaw but keep on ice.
2. Take the following out of 4C fridge to come to room temperature for pipetting:
    - 2mg/ml BSA stock solution (Sigma P0834-10X1ML).
    - Tyrode's buffer or whichever solution tissue is suspended in for dilutions
    - Bradford reagent
3. Keep original samples on ice. Standards and diluted samples are better to be left at room temperature for pipetting.
4. Gather 1.5 ml centrifuge tubes, as many needed for 7 standards and all samples.
5. Label tubes with standard concentrations or sample numbers (with dilution factor). Use as a guide for sample dilutions: serum 200X, urine 10X, kidney homogenates 20X. Dilutions may not be this high. (see instructions below for serial dilution).
6. Make standard dilutions using BSA stock solution. Transfer with a pipette into a 2.0mL microcentrifuge tube. Standards should range in concentration from 1mg/ml to 0.1mg/ml. Remember to make enough needed for next serial dilution and triplicate samples. The concentrations will be 1mg/mL, 0.75mg/mL, 0.56mg/mL, 0.42mg/mL, 0.32mg/mL, 0.24mg/mL, & 0.12mg/mL. The curve will not be linear for standards lower than 0.12mg/ml, so do not make concentrations lower than this. Vortex solutions after mixing and before pipetting out for next serial dilution.

7. Prepare diluted samples according to step #5. example.: 10µl of kidney homogenate sample with 190µl of Tyrode's (pH 7.6)/ultrapure H2O = 20x dilution. Vortex samples after diluting.
8. Label a template guide with the position of the blank, standards and samples.
9. Vortex standards and diluted samples directly before pipetting onto plate. Working in triplicate, pipette 10µl of water/Tyrode's buffer in wells A1, A2, and A3 of plate (Costar, 3368). These are the blanks. Pipette standards and samples in the same way.
10. Swirl bottle of Bradford dye (Bradford Reagent, Sigma B6916) gently and fill reagent reservoir, pouring out only what is needed. Using a 300µl multichannel pipette, pipette 200µl of Bradford into each well and mix contents with the pipette (max 10 min for this step). Cover plate with plate cover and incubate plate on rocker or orbital shaker for 15 minutes at room temperature. Shake or rotate gently. The protein-dye complex is stable up to 60 minutes.
11. The wavelength for reading the plate is 595nm. Set up plate reader using the GENLAB software to the correct plate template while plate is incubating. Time is IMPORTANT. Do not let the samples incubate longer than 15 minutes (If the plate is incubated longer, the color will be darker, therefore skewing protein concentrations). Bring plate to plate reader before 15 minutes are up so that plate can be read at exactly 15 min.

Instructions for Serial Dilution

1. Obtain 7 microfuge tubes and label them with the following standard concentrations: 1mg/mL, 0.75mg/mL, 0.56mg/mL, 0.42mg/mL, 0.32mg/mL, 0.24mg/mL, & 0.12mg/mL.

2. Add water/buffer (depending on what your samples are suspended in) to the tubes as follows:

   1 = 100 µL
   2 = 50 µL
   3 = 50 µL
   4 = 50 µL
   5 = 50 µL
   6 = 50 µL
   7 = 150 µL

3. Vortex 2mg/mL stock BSA and add **100 µL** to tube 1 and vortex.

4. Add 150 µL of tube 1 to tube 2 and vortex

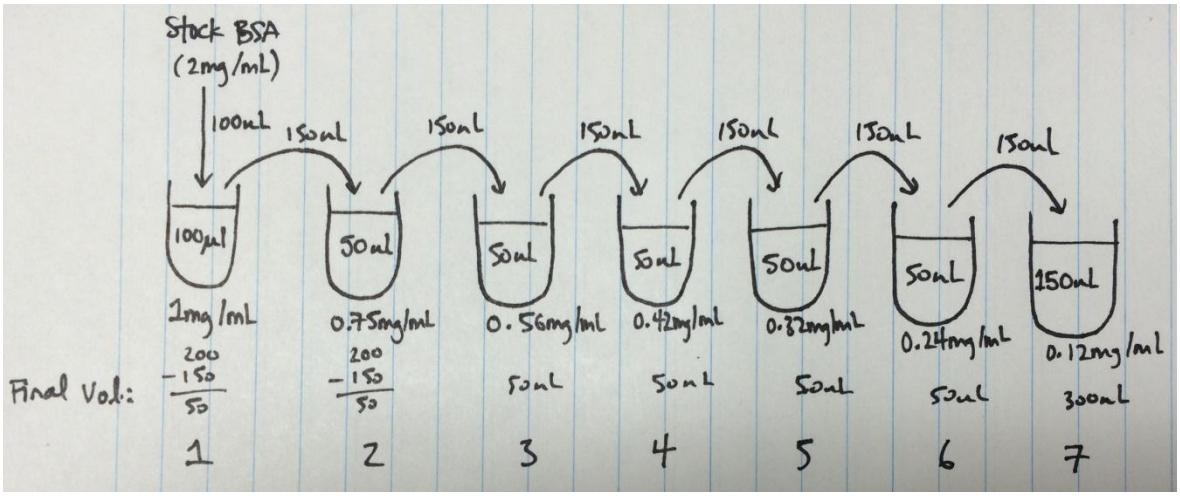
5. Add 150 µL of tube 2 to tube 3 and vortex

6. Add 150 µL of tube 3 to tube 4 and vortex.

7. Add 150 µL of tube 4 to tube 5 and vortex.

8. Add 150 µL of tube 5 to tube 6 and vortex.

9. Add 150 µL of tube 6 to tube 7 and vortex.



Appendix C:

Methods for creatinine assay – urine and serum

Reference: Heinegard D, Tiderstrom G. 1973 Determination of serum creatinine by a direct colorimetric method. (Jaffe reaction, 1928) Clinica Chimica Acta 43: 305-310.

Solutions (prepare in advance & keep as stock):

(a) **0.05 M Sodium Phosphate and 0.05 M Sodium Borate** (*keep refrigerated*)

- Add 13.40 g of $Na_2HPO_4 \cdot 7H_20$ (sodium phosphate) and 6.9 g of $BNaO_2 \cdot 4H_20$ (sodium borate) to 900 mL of distilled water
- Add 10 M aqueous sodium hydroxide (drop by drop) to reach a pH of 12.7
- Top up solution with distilled water to 1 L

(sodium phosphate 268.07 g = 1 mole (sodium borate 137.9g = 1 mole

13.4035 g = 0.05 mole) 6.895g = 0.05 mole)

**Na_2HPO_4 can be used if $Na_2HPO_4 \cdot 7H_20$ is not available.

(Na_2HPO_4 142 g = 1 mole

7.1 g = 0.05 mole)

To make 10 M Sodium Hydroxide:

(Sodium Hydroxide 40g = 1 mole

400 g = 10 mole)

For example: to make 100 ml 10 M sodium hydroxide: dissolve 40g sodium hydroxide in small amount of distilled water (ie 80 ml) in a beaker, and then transfer sodium hydroxide solution into a 100 ml volumetric flask and add distilled water to reach the line.

(b) 4% aqueous SDS (room temperature)

- Add 40 g SDS to 1 L distilled water

* SDS is a detergent and therefore can become bubbly very easily; swirl to mix

(c) 60% Acetic Acid solution (room temperature)

- 60 mL concentrated Acetic Acid + 40 mL distilled water

(c) Picric Acid 1.3% (room temperature) (Sigma, cat. # P6744-1GA)

Make Fresh Daily:

(a) Picric solution **(Combine in proportions of 2:2:1)**

- 2 volumes 0.05 M Sodium Phosphate and 0.05 M Sodium Borate solution (a)
- 2 volumes of 4% aqueous SDS (b)
- 1 volume Picric Acid (d)

For example: To make 25 mL (enough for one 96 well plate)

10 ml 0.05 M Sodium Phosphate and 0.05 M Sodium Borate solution (a)

10 ml 4% aqueous SDS (b)

5 ml Picric Acid (c)

*Swirl to mix

(b) 15% Acetic Acid solution (prepare using a 1:4 dilution)

- 1 mL 60% Acetic Acid
- 3 mL Picric solution

Procedure

Step 1: Prepare standards, samples, and reagents

Standards: (make new for each plate)

Use standard set from Sigma (cat. # C3613) *See note on bottom of last page

contains stock concentrations of: 0.01mg/ml, 0.03mg/ml, & 0.10mg/ml

For Urine – use standard concentrations of:

0.005mg/ml, 0.01 mg/ml, 0.02 mg/ml,0.03 mg/ml, 0.05mg/ml, 0.06mg/ml, 0.08mg/ml, & 0.10mg/ml

For Serum – use standard concentrations of 0.0025mg/ml, 0.050mg/ml, 0.01mg/ml, 0.03mg/ml, 0.05mg/ml, and 0.06mg/ml (0.08mg/ml and 0.10mg/ml can also be prepared to increase accuracy of standard curve).

Samples:

Urine – Dilutions vary by study (Undiluted to 100-fold dilution)

*Rat samples often require a 20-fold dilution

Serum – No dilution required

Reagents:

Prepare picric solution and 15% acetic acid solution as directed above

Step 2: Plate 20 µl of blank (deionized water), standards, and samples to wells of 96 well-plate (Fisher, cat # 07-200-641). Make duplicates or triplicates of each.

Step 3: Add 200 µl of Picric Solution to each well using a multi-tip pipet with yellow tips (and mix by filling and expelling tips once.

(There may be a few bubbles present due to SDS, this is not a problem during the incubation, however be use that the bubbles are popped before reading plate/)

Step 4: Cover plate with well cover sheet (Cayman, cat # 400012) and incubate at room temperature on orbital shaker at 140rpm or Vortex-Genie 2 at lowest speed for 40 minutes.

After 40 minutes, remove well cover sheet (this will cause the bubbles to disappear) and place well-plate on the microplate reader and shake every 15-20 seconds for 5 seconds for the last 3-4 minutes.

Step 5: Read 96 well-plate at 500 nm on microplate reader. Print and save results.

Step 6: Add 20 µl of 15% Acetic Acid solution using a multi-tip pipet (make sure pipet tips touch the bottom of each well) and mix by filling and expelling the tip once

Step 7: Incubate at room temperature for 6 minutes (begin timing as soon as 15% Acetic Acid solution is added to the last row of wells). To ensure adequate mixing, place well-plate on the microplate reader and shake every 15-20 seconds for 5 seconds for the last 3-4 minutes.

Step 8: Read well-plate at 500 nm on microplate reader. Print and save results

Step 9: Calculations (Use Microsoft Excel spread sheet)

a) To find the final absorbance of standards and samples:

Final absorbance = pre-acid absorbance values – post-acid absorbance values

b) Take average of results (any CV less than 10% is acceptable)

c) Construct a standard curve with absorbance on the x-axis and concentration on the y-axis. Using the equation given from this curve, calculate the concentration of creatinine in urine and/or serum samples. (If samples were diluted, multiply concentration by the dilution factor.)

Appendix D:

Methods for histological analysis

Step A: Acquiring pictures of whole kidney

- Pictures of whole kidney sections are taken to ensure complete coverage of the cyst area.
- Mount the Nikon DSLR camera on the tripod.
- Connect 60mm macro lens, and use spacers (teleconverter) between the camera and lens to achieve maximum magnification.
- Invert the camera with lens facing down
- Keep the slide on a back lit surface under the lens (about 10 cm away)
- Focus the camera on the tissue by half pressing the button
- Once focus is achieved, turn the focus switch to manual focus position.
- Take pictures of all the kidneys from the same postion. Be careful not to change the distance between the lens and the slide, as this will change the magnification.
- Take a picture of a micrometer under the same conditions, to convert pixel dimensions to length later on.

Step B: Measuring cyst area from whole kidney pictures

- Open Image-Pro Plus Software 6.0, Select 'Complete' Select 'Done'
- First you must create a colour standard. To do this open a kidney photograph that has a lot of cysts. Click the measure tab and then click "count/size" Click "select colours"
- Under the colour cube based tab click the left button that looks like a pen. Now put the mouse over your kidney photograph and select the truest colours representing cyst area. Look at the colour representation cube on the count/size window to better tell the difference

between colours. Once you're happy with your colour selection, click File.

- Click Save file and save your colour standard under a recognizable name. You must load this standard for every picture you analyze.

Now open your kidney picture.

- Under the measure tab, click the data collector.
- Under the layout tab, select "image" in the first drop down box. Then select name and the click the right arrow to add it to the right.
- Where it says "image" in the drop down box, select "count/size" then select "area" from the list below.
- The dropdown box below select sum, then click the arrow to add it to the list to the right. Click the top tab labelled "Data list". This is where your data will be placed. Now, close the "segmentation – image x" window. In the count/size window click "select colours"
- Click "File"
- Click "Load File"
- Select the colour standard that you just created
- Click "yes" to replace the ranges.
- Close the segmentation window
- One the count/size window, select "count"
- In the data collector window, select "collect now".
- The area sum of the cyst area from your photo should appear in the data list.
- Repeat for all kidneys. Once finished, click the "export" tab in the data collector window.

- Select export target
- Select export now. Your data will be exported to the active excel sheet. To change the export options, click “export options”.

  **If you wish to exclude certain elements of your photograph such as tubular lumen, you can simply crop it out using paint and open the modified picture in the Image pro plus software.

Appendix E:

Methods for oxylipin analysis

Step A: Measuring Kidney Tissue and Tyrode's

Use Tyrode's (pH 7.6) salt solution to homogenize dried kidney tissue. For every 70 mg of dried (lyophilized) kidney tissue approximately 2000uL of Tyrode's (pH7.6) is required.

Therefore, 45 mg of dry (lyophilized) tissues = 1250 uL of Tyrode's required

Step B: Homogenizing

1. Ensure there is enough prepared of:
    - Tyrode's salt solution (pH 7.6) (Check for deterioration.See Solutions Preparation below)
    - 1.4X whole cell buffer – only if doing westerns (see western blotting buffer solutions)
    - 1% Triton Solution as per instructions below (see Solutions Preparation).
    - 12 mL test tubes with lids that have been soaked overnight in Contrad solution, rinsed and dried.
    - 100:1 Methanol and Formic Acid
    - pH 3 water – (water that has had pH adjusted to 3.0 using 1M HCL)
    - Antioxidant Cocktail
2. Obtain a large container of ice
3. Remove the required lyophilized kidney samples from the -80C freezer and keep on ice.
4. Label 16 x 125mm disposable glass test tubes with sample ID's
5. If samples appear to be a fine powder proceed to weighing. If there are clumps in the sample, pulverize before weighing out sample.

6. Weigh and record **45 mg** (can be +/- 2 mg – just make sure that the weight is recorded) lyophilized kidney sample into labeled tubes (prepared in step 4), cover with parafilm and immediately place on ice.
7. Calculate, record, and add required amount of Tyrode's (pH7.6) to each massed kidney sample.

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

8. Prepare and label three disposable glass tubes (16 x 125mm) with 100% ethanol and three disposable glass tubes (16 x 125mm) with ultrapure water for cleaning the homogenizer
9. Clean homogenizer before use, after use, and in between each sample by:
    - 3 tubes ethanol x 30 seconds each at speed 15
    - 3 tubes ultrapure water x 30 seconds each at speed 15
    - dab with kimwipe to dry
10. Place test tube containing lyophilized kidney tissue in a small plastic container containing an ice slurry (ice plus water).
11. Insert rotor into test tube and homogenize at speed 20 for 30 seconds. Avoid generating bubbles. Stop and check that all kidney tissue is at the bottom of the tube. If not, use rotor tip to push everything to the bottom of the test tube.
12. Homogenize again for another 30 seconds (speed 20).
13. Repeat steps 10 – 13 for each sample. Remember to keep tubes covered with parafilm and on ice as much as possible

Step C: Aliquoting homogenate

From each sample, remove an aliquot of 200 uL for the oxylipin analysis and place in a microcentrifuge tube. Go to Step D for further preparation.

Step D: Preparation of fraction for solid phase extraction of Oxylipins

1. Add 1 uL of a 1% Triton solution for every 100 uL aliquot for oxylipin analysis. (Final concentration of Triton in homogenate should be 0.01%: 0.01x 100=1ul)
2. Vortex for 10 seconds
3. Incubate, covered, on ice for 10 minutes
4. Vortex again for 10 seconds
5. Incubate, covered, on ice for 10 minutes
6. Vortex again for 10 seconds
7. Incubate, covered, on ice for 10 minutes
8. For the oxylipin fraction, vortex and aliquot 200 uL of tyrode's homogenate into two labeled 2 mL microtubes.
9. Vortex each 2 mL microtube for 10 seconds.
10. Working quickly, add in the same order below to the samples;

a. 500 uL of 100:1methanol formic acid

b. 800 uL of pH3 water

c. 90 uL of 100% ethanol

d. 10 uL of antioxidant cocktail

e. Vortex for 5 seconds.

11. Store samples in -80C freezer for future oxylipin extraction – see step E.

Step E: Solid phase extraction of eicosanoids

Ensure that there is enough of the following:

- Internal Standard
- Strata-X SPE columns (CAT# 8B-S100-UBL)
- pH3 water
- methanol (neat)
- 10% methanol in pH3 water
- Solvent A

*Samples should be kept on ice whenever possible.

***Do not** allow the column to run dry during steps 12,13, or 14

Sample Preparation for Oxylipin Analysis

1. Remove homogenized samples from -80°C freezer and defrost on ice.
2. Cool-down centrifuge to 4C.
3. Turn on nitrogen evaporator water bath to 37C. Make sure evaporator has enough water in it, if not add distilled water.
4. Add 1mL of pH3 water to a small test tube. One thawed vortex and transfer 1ml of the sample into the test tube.
5. Add 10 uL of internal standard (kept in -20 freezer) to each glass tube. Vortex.
6. Acidify samples to pH 3 with 1N HCl if necessary. Use pH-indicator strips to test pH.

7. Centrifuge for 5 min at 3000 rpm at 4C to remove debris
8. In the fume hood, set-up and label a Strata-X SPE (Phenomenex) (33u, 60 mg/3mL) column for each sample using the wooden rack designed for columns. The SPE columns are stored in the dessicator.
9. Place a waste vial under each column.
10. Precondition column by adding 2000 uL of 100% methanol. Let methanol drip into waste container by gravity for 1 min, then you may gently use the BD 10 mL syringe (cat# 148232a) to help push methanol through column (only push a few drops through at a time).
11. Precondition column by adding 2000 uL of pH 3 water. Let pH 3 water drip through the same way you did in step #12 with the methanol.
12. Apply sample to column, avoiding debris in the bottom of the tube. Let drip into waste container by gravity.
13. Add 1000 uL 10% methanol in pH3 water to emptied sample tube. Vortex, centrifuge, and apply to column as the wash. Push through to dry column.
14. Elute into new, labeled 1.5 mL microtubes. To elute, add 1000 uL of 100% methanol to column and allow to drip through by gravity (and soak into column) for at least 1 min. Then gently and slowly push through with syringe. Once run through, run column dry for 30 seconds by pushing through using the BD 10 mL syringe.
15. If the samples are not being run that day on the LCMS, displace air with nitrogen gas and store at -80C until the day they will be run. Then dry down.
16. Drying down in the nitrogen evaporator:
    a. Set the water bath in the nitrogen evaporator to 37C.

b. Open one or two of the needles
c. Open the valves and adjust the amount of nitrogen gas coming out of the needles with the knob that is attached to the side of the water bath (LPM AIR)
d. Open a needle for each tube that you will be drying.
e. Clean the needle with 100% chloroform. Dip the needles into the chloroform. You can watch the bubbles to see how strong your flow of nitrogen gas is.
f. Put your tubes into the evaporator, and lower the needles so that they are *gently* blowing nitrogen gas on the surface of the solution in the tube. Do not let the needles touch the solution.
g. Leave your samples to dry for about an hour. Check on them every 15 minutes to ensure the temperature is kept at 37C. As the samples evaporate you can lower the needle.

17. Adding Solvent A (water-acetonitrile-formic acid [70:30:0.02 v/v/v] *LC-MS Grades):
    a. Once the samples have dried (there should be no methanol left in the tubes, just dried residue from the sample), take them out of the water bath.
    b. Take the solvent A out of the fridge (4C) and add 100 uL to each tube.
    c. Vortex each tube so that all the dried sample is mixed in with the solvent A.
    d. Centrifuge the samples at 14000g (rcf) for 5 minute at 4C.
    e. Transfer into labeled GC/LC vials containing a 200ul Target polypropylene conical insert.
    f. Run on LCMS the same day.

Preparation of solutions

To Reconstitute Tyrode's Salts Without Sodium Bicarbonate

Product# T2145 (Sigma)

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

1. Measure out 1L of ultrapure water in a plastic graduated cylinder. Water temperature should be 15-20C
2. Transfer about 800 mL of measured water into a 2000 mL flask. Add a large stir bar and place on stir plate. Begin gentle stirring.
3. Add powdered Tyrode's salts and continue stirring until dissolved. Do NOT heat.
4. Rinse original Tyrode's salts packaging with some of the remaining 200 mL measured water to remove all traces of powder. Add to solution in step 3. Rinse the package 3 times.
5. Transfer solution to a 1L volumetric flask and bring to volume using some of the remaining 200 mL measured water in graduated cylinder from step 1. Rinse the flask 3 times. Can use a pipette to bring the flask up to volume w/ water.
6. Insert volumetric stopper and invert 10x to mix. Before inverting, ensure the stopper and mouth of the volumetric flask are completely dry to prevent leaks.
7. Transfer to a 1L glass bottle covered with tin foil to protect from light and clearly label as Tyrode's salts WITHOUT NaHCO3. Store in the refrigerator (2-8C).

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

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

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

- pH change
- precipitate or particulates
- cloudy appearance

- colour change

Check all these signs before using. Use Tyrode's (pH 7.6) to make 1% Triton.

To Make 1% Triton

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

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

A final concentration of 0.01% Triton is required in the homogenate to disrupt lipids and release proteins. This will ONLY be added to the LC-MS/MS fraction. Therefore, add 10uL of 1% Triton (pH 7.6) to 1000uL of kidney homogenate for the LC-MS/MS fraction only to make a 0.01% Triton final solution

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

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 MeOH:EtOH:H_2O).

Make a minimum of 100 mL Antioxidant Cocktail. Measure out 50 mL of Methanol and 25ml Ethanol in separate graduated cylinders. Mix together in a 250 mL beaker. Cover the outside of

the beaker with tinfoil and place on magnetic stirrer. Put stir bar in beaker and cover the top with tinfoil to minimize volatilization. Weigh out 20 mg BHT, 20 mg EDTA, 200 mg TPP and 200 mg Indomethacin onto separate weigh paper. Add the ingredients to the MeOH:EtOH solution and stir solution until all dissolved. This will take a while. Keep beaker completely covered with tinfoil to also minimize exposure to light. When all dry ingredients are dissolved, transfer the mixture to a 100ml volumetric flask. Using a small amount of ddH2O, wash down the sides of the beaker and pour into the 100ml flask. Do this 3 times to ensure you transfer all of the solvent and antioxidants. Fill up the 100ml volumetric flask to the mark with ddH_2O. Stopper flask and invert 10x to mix. Transfer into a clean, tinfoil covered, labeled 125 mL bottle. Aliquot the appropriate amount of antioxidant cocktail into covered scintillation vials for individual users.

Solvent A

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

*MS Grade

*Prevent evaporation of prepared solutions using paraffin around cap seals

To make 1000 mL:

700 mL water

300 mL acetonitrile

200 uL acetic acid

Vacuum filter through Whatman #4 filter paper

Solvent B

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

*MS Grade

To make 1000mL:

500 mL Acetonitrile

500 mL Isopropyl alcohol

Vacuum filter through Whatman #4 filter paper

Appendix F:

List of oxylipins scanned for, internal standard used and dose response factors

Oxylipin	Internal Standard	Detector Response Factor
LA derived oxylipins		
9-HODE	9-HODE-d4	1.5356
9-oxoODE	5-oxoETE-d7	1.2722
9,10,13-TriHOME	9,10 diHOME-d4	1.4432
9,12,13-TriHOME	12,13 diHOME-d4	0.4625
9,10-EpOME	9,10 EpOME-d4	0.9435
9,10-DiHOME	9,10 diHOME-d4	2.7837
13-HODE	13-HODE-d4	1.4445
13-oxo-ODE	5-oxoETE-d7	3.8609
12,13-EpOME	12,13 diHOME-d4	0.3934
12,13-DiHOME	12,13 diHOME-d4	2.3577
GLA derived oxylipins		
13-HOTrE-y	13-HODE-d4	1.278
DGLA derived oxylipins		
PGD_1	PGD_2-d4	0.2034
PGE_1	PGE_2-d4	0.3064
PGF_{1a}	PGF_{2a}-d4	3.4537
PGK_1	PGE_2-d4	
TXB_1	TXB_2-d4	1.2753
8-HETrE	5-HETE-d8	1.175
15-HETrE	15-HETE-d8	2.2989
15-keto-PGF_{1a}	PGF_{2a}-d4	
AA derived oxylipins		
bicyclo-PGE_2	PGE_2-d4	0.0967
dihydro-PGF_{2a}	PGF_{2a}-d4	0.934
dihydro-keto-PGD_2	PGD_2-d4	0.7189
dihydro-keto-PGE_2	PGE_2-d4	0.117

dihydro-keto-PGF_{2a}	dihydro-keto-PGF2a-d4	1.37188
HXA_3	LTB_4-d4	
HXB_3	LTB_4-d4	
LTB_4	LTB_4-d4	0.1936
LTC_4	LTB_4-d4	
LTD_4	LTB_4-d4	
LTE_4	LTB_4-d4	
LXB_4	LTB_4-d4	0.8955
PGA_2	15 deoxy-PGJ_2-d4	0.4698
PGB_2	15 deoxy-PGJ_2-d4	0.0289
PGD_2	PGD_2-d4	0.8821
PGE_2	PGE_2-d4	1.6058
PGF_{2a}	PGF_{2a}-d4	2.2234
PGJ_2	15d PGJ_2-d4	0.331
PGK_2	PGE_2-d4	0.9502
tetranor-12-HETE	15-HETE-d8	1.1197
tetranor-PGDM	PGD_2-d4	
tetranor-PGEM	PGE_2-d4	0.146
tetranor-PGFM	PGF_{2a}-d4	
TXB_2	TXB_2-d4	1.5179
2,3 dinor-11b-PGF_{2a}	PGF_{2a}-d4	5.0098
2,3 dinor-8-iso-PGF_{2a}	PGF_{2a}-d4	
2,3 dinor-TXB_2	TXB_2-d4	
2,3 dinor-6k-PGF_{1a}	6 keto-PGF_{1a}-d4	
5-HETE	5-HETE-d8	1.1522
5-oxoETE	5-oxoETE-d7	2.3064
5-iso-PGF_{2a}VI	PGF_{2a}-d4	2.8143
5,15-DiHETE	LTB_4-d4	1.4967
5,6-DiHETE	LTB_4-d4	0.00205
5,6-DiHETrE	11,12 DiHETrE-d11	0.6898
5,6-EpETrE	11,12 DiHETrE-d11	
6 keto-PGE_1	PGE_2-d4	1.0954

6 keto-PGF_{1a}	6 keto-PGF_{1a}-d4	1.8497
6R-LXA_4	LTB_4-d4	0.9549
6S-LXA_4	LTB_4-d4	
6 trans-LTB_4	LTB_4-d4	0.7172
6 trans,12epi-LTB_4	LTB_4-d4	1.0461
6,15 diketo-,dihydro-	PGF_{2a}-d4	2.9725
8-HETE	5-HETE-d8	0.7561
8,15-DiHETE	LTB_4-d4	0.6456
8,9-DiHETrE	8,9 DiHETrE-d11	1.519
8,9-EpETrE	8,9 DiHETrE-d11	
8 iso-15k-PGF_{2a}	PGF_{2a}-d4	
8 iso-PGF_{2a}III	PGF_{2a}-d4	0.8407
9-HETE	5-HETE-d8	0.3572
11-HETE	5-HETE-d8	4.1226
11,12-DiHETrE	11,12 DiHETrE-d11	1.3714
11,12-EpETrE	11,12 DiHETrE-d11	
11 beta-PGF_{2a}	PGF_{2a}-d4	0.9436
11 beta-dihydro-keto-	PGF_{2a}-d4	2.6124
11 beta-PGE_2	PGE_2-d4	0.5271
11dehydro-TXB_2	TXB_2-d4	0.0521
12-HETE	15-HETE-d8	1.7272
12-HHTrE	15-HETE-d8	0.4246
12-oxoETE	5-oxoETE-d7	11.5195
12epi-LTB_4	LTB_4-d4	2.0533
12-oxo-LTB_4	LTB_4-d4	1.4203
14,15-DiHETrE	14,15 DiHETrE-d11	1.1769
14,15-EpETrE	14,15 DiHETrE-d11	
14,15-LTC_4 (EXC_4)	LTB_4-d4	
14,15-LTD_4 (EXD_4)	LTB_4-d4	
14,15-LTE_4 (EXE_4)	LTB_4-d4	
15-HETE	15-HETE-d8	1.5717
15-oxoETE	5-oxoETE-d7	5.8769

15 deoxy-PGA_2	15 deoxy-PGJ_2-d4	0.0478
15 deoxy-PGD_2	15 deoxy-PGJ_2-d4	1.3276
15 deoxy-PGJ_2	15 deoxy-PGJ_2-d4	1.597
15 keto-PGD_2	PGD_2-d4	
15 keto-PGE_2	PGE_2-d4	0.5272
15 keto-PGF_{2a}	PGF_{2a}-d4	1.3536
15R-LXA_4	LTB_4-d4	
16-HETE	15-HETE-d8	2.0377
17-HETE	15-HETE-d8	
18-HETE	15-HETE-d8	1.4061
19-HETE	20-HETE-d6	0.8117
19 hydroxy-PGE_2	PGE_2-d4	0.2223
19 hydroxy-PGF_{2a}	PGF_{2a}-d4	
20 hydroxy-LTB_4	LTB_4-d4	
20-HETE	20-HETE-d6	1.3024
20 carboxy-AA	ARA-d8	
20 carboxy-LTB_4	LTB_4-d4	0.2898
20 hydroxy-PGE_2	PGE_2-d4	
20 hydroxy-PGF_{2a}	PGF_{2a}-d4	
ADA derived oxylipins		
dihomo-15 deoxy-PGD_2	15 deoxy-PGJ2-d4	
dihomo-PGD_2	PGD_2-d4	
dihomo-PGE_2	PGE_2-d4	
dihomo-PGF_{2a}	PGF_{2a}-d4	
dihomo-PGJ_2	15 deoxy-PGJ_2-d4	
ALA derived oxylipins		
9-oxoOTrE	5-oxoETE-d7	2.396
9-HOTrE	9-HODE-d4	1.342
9,10-diHODE	9,10 diHOME-d4	
9,10-EpODE	9,10 diHOME-d4	
12,13-DiHODE	12,13 diHOME-d4	0.6371
12,13-EpODE	12,13 diHOME-d4	0.255

13-oxoOTrE	5-oxoETE-d7	0.2949
13-HOTrE	13-HODE-d4	0.0042
15,16-DiHODE	12,13 diHOME-d4	
15,16-EpODE	12,13 diHOME-d4	
EPA derived oxylipins		
Δ17-6 keto-PGF_{1a}	PGF_{2a}-d4	12.354
LXA_5	LTB_4-d4	0.0268
PGD_3	PGD_2-d4	0.1468
PGE_3	PGE_2-d4	0.669
PGF_{3a}	PGF_{2a}-d4	1.1279
RvE_1	LTB_4-d4	
TXB_3	TXB_2-d4	1.6368
5-HEPE	5-HETE-d8	0.8279
8-HEPE	5-HETE-d8	0.5926
8 iso-PGF_{3a}	PGF_{2a}-d4	
9-HEPE	5-HETE-d8	0.3766
11-HEPE	5-HETE-d8	0.7798
12-HEPE	15-HETE-d8	1.4911
14,15-EpETE	14,15 DiHETrE-d11	
15-HEPE	15-HETE-d8	0.4255
15-oxoEDE	5-oxoETE-d7	0.9284
17,18-EpETE	14,15 DiHETrE-d11	
18-HEPE	20-HETE-d6	0.6622
DPA derived oxylipin		
17 keto-DPA/17oxo-DPA	LTB_4-d4	
DHA derived oxylipins		
4-HDoHE	5-HETE-d8	1.3963
7-HDoHE	5-HETE-d8	1.0288
7R Maresin-1	LTB_4-d4	0.0499
8-HDoHE	5-HETE-d8	0.4553
PD_1	LTB_4-d4	
RvD_1	LTB_4-d4	0.6921

RvD_2	LTB_4-d4	0.5169
10-HDoHE	5-HETE-d8	2.4108
10S,17S-DiHDoHE (PDX)	LTB_4-d4	2.7598
11-HDoHE	5-HETE-d8	1.7831
13-HDoHE	15-HETE-d8	3.104
14-HDoHE	15-HETE-d8	1.0587
15 trans-PD1	LTB_4-d4	
16-HDoHE	15-HETE-d8	3.1309
16,17-EpDPE	14,15 DiHETrE-d11	0.0165
17-HDoHE	15-HETE-d8	0.318
17 keto-DHA / 17 oxo-	LTB_4-d4	0.536
19,20-DiHDPE	14,15 DiHETrE-d11	0.2102
19,20-EpDPE	14,15 DiHETrE-d11	0.1305
20-HDoHE	20-HETE-d6	1.1182
MA derived oxylipins		
5-HETrE	5-HETE-d8	1.2132
OA derived oxylipins		
10-Nitrooleate	ARA-d8	
9-Nitrooleate	ARA-d8	

Abbreviations: AA, Arachidonic acid; ADA, Adrenic acid; ALA, Alpha-linolenic acid; DGLA, Dihomo-gama-linolenic acid; DHA, Docosahexaenoic acid; DiHDoHE, Dihydroxy-docosahexaenoic acid; DiHDPE, Dihydroxy- docosapentaenoic acid; DiHEPE, 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; DPA, Docosapentaenoic acid; EpDPE, Epoxy-docosapentaenoic acid; EpEDE, Epoxy-eicosadienoic acid; EpETE, Epoxy-eicosatetraenoic acid; EpETrE, Epoxy-eicosatrienoic acid; EpODE, Epoxy-octadecadienoic acid; EpOME, Epoxy-octadecenoic acid;

EPA, Eicosapentaenoic acid; Ex, Eoxin; GLA, Gama-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; HpODE, Hydroperoxy-octadecadienoic acid; Hx, Hepoxilin; LA, Linoleic acid; Lt, Leukotriene; Lx, Lipoxin; MaR, Maresin; MA, Mead acid, OA, Oleic acid; oxo-EPE, oxo-Eicosapentaenoic acid; oxo-ETE, oxo-Eicosatetraenoic acid; oxo-ODE, oxo-Octadecadienoic acid; oxo-OTrE, oxo-Octadecatrienoic acid; PD, Protectin; PG, Prostaglandin; PGEM, Prostaglandin E metabolite; Rv, Resolvin; TriHOME, Trihydroxy-octadecenoic acid; Tx, Thromboxane.

Note: Detector response factors and retention times could not be experimentally determined for those oxylipins where primary standards were not commercially available. In such instances, retention times from the following 2 publications were used for screening these oxylipins.

[1]. Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA.High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acylethanolamines. Biochim Biophys Acta. 2011 Nov;1811(11):724-36. doi: 10.1016/j.bbalip.2011.06.005.

[2]. 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. J Chromatogr A. 2014 Sep 12;1359:60-9. doi: 10.1016/j.chroma.2014.07.006.