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Placental Eicosanoids and Sphingolipids in Preeclampsia

Daniel T. Reep
Virginia Commonwealth University

PLACENTAL EICOSANOIDS AND SPHINGOLIPIDS IN PREECLAMPSIA

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science at Virginia Commonwealth University.

by

Daniel Tiberius Reep
Bachelor of Science, Biomedical Engineering, Virginia Commonwealth University, 2015

Director:
Scott W. Walsh, Ph.D.
Professor, Departments of Obstetrics & Gynecology and Physiology & Biophysics
Virginia Commonwealth University, School of Medicine

Virginia Commonwealth University
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Abbreviations Used

11,12-DHET .....11,12-Dihydroxyeicosatrienoic Acid
14,15-DHET .....14,15-Dihydroxyeicosatrienoic Acid
14,15-EET .....14,15-Epoxyeicosatrienoic Acid
15d-PGJ_2 .....15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2
6-keto-$PGF_{1\alpha}$ .....6-keto Prostaglandin F_{1alpha}
8,9-DHET .....8,9-Dihydroxyeicosatrienoic Acid
8,9-EET .....8,9-Epoxyeicosatrienoic Acid
8-iso $PGF_{2\alpha}$ .....8-iso Prostaglandin F_{2alpha}
AA .....Arachidonic Acid
ACOG .....American College of Obstetricians and Gynecologists
ASA .....Aspirin (Acetylsalicylic Acid)
BCA .....Bicinchoninic Acid
BSA .....Bovine Serum Albumin
C1P Ceramide-1-Phosphate
COX .....Cyclooxygenase
COX-1 .....Cyclooxygenase-1
COX-2 .....Cyclooxygenase-2
DHA .....Docosahexaenoic Acid
DHET .....Dihydroxyeicosatrienoic Acid
DHGLA .....Dihomo-Gamma-Linolenic Acid
EET .....Epoxyeicosatrienoic Acid
EOPE .....Early Onset Preeclampsia
EPA .....Eicosapentaenoic Acid
ESI-MS .....Electrospray Ionization Mass Spectrometry
HETE .....Hydroxyeicosatetraenoic Acid
LOPE .....Late Onset Preeclampsia
LTA_4 .....Leukotriene A_4
LTB_4 .....Leukotriene B_4
LTC_4 .....Leukotriene C_4
LTD_4 .....Leukotriene D_4
LTE_4 .....Leukotriene E_4
mcg .....Microgram

mg .....Milligram
mL.....Milliliter
ng.....Nanogram
NP.....Normal Pregnancy
PBS .....Phosphate Buffered Saline
PE.....Preeclamptic, Preeclampsia
pg.....Picogram
PGA_2 .....Prostaglandin A_2
PGD_2 .....Prostaglandin D_2
PGE_1.....Prostaglandin E_1
PGE_2.....Prostaglandin E_2
$PGF_{1\alpha}$ .....Prostaglandin F_{1alpha}
$PGF_{2\alpha}$ .....Prostaglandin F_{2alpha}
PGI_2.....Prostaglandin I_2 (Prostacyclin)
PGJ_2 .....Prostaglandin J_2
pmol .....Picomole
PUFA.....Polyunsaturated Fatty Acid
S1P.....Sphingosine-1-Phosphate
TXA_2.....Thromboxane A_2
TXB_2 .....Thromboxane B_2
VCU.....Virginia Commonwealth University
xg .....Times Acceleration of Gravity (Relative Centrifugal Force)
µL.....Microliter

Abstract

PLACENTAL EICOSANOIDS AND SPHINGOLIPIDS IN PREECLAMPSIA

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Placental dysfunction is implicated in the pathogenesis of preeclampsia. Chemical signals between the placenta and maternal circulation are a suspect cause of endothelial dysfunction and maternal hypertension. This study examined select lipid mediators of inflammation produced by the placenta. Patients were recruited from Virginia Commonwealth University's pregnancy clinics and placentas were collected at delivery. Forty-eight-hour explant cultures of villous placental tissue were used to model lipid production. Electrospray ionization

mass spectrometry was used to quantify concentrations of free lipids in the culture media. Bicinchoninic acid assays were performed to quantify protein in each culture for normalization of lipid data. After analysis, it was found that severity of preeclampsia was correlated with a unique lipid profile. Pro-inflammatory hydroxyeicosatetraenoic acids and sphingolipids were elevated. Aspirin usage in patients who developed preeclampsia was found to attenuate accumulation of isoprostane oxidative stress markers and thromboxane production while preserving omega-3-fatty acid and increasing prostacyclin levels.

Chapter 1: Introduction

A. General Background

a. Preeclampsia and the Placenta

Preeclampsia (PE) is a pathological condition in pregnancy characterized by hypertension and proteinuria with progressive effects on multiple organ systems [1, 2]. PE affects approximately 4.6% of pregnancies worldwide, with increased incidence in first pregnancies [3]. Additionally, 10-15% of maternal deaths involving pregnancy complications involve PE [3]. Other severe features of PE include thrombocytopenia, impaired liver function, renal insufficiency, and pulmonary edema [2]. As of 2013, diagnostic criteria are based on blood pressure readings $\geqq$ 140 mmHg systolic or $\geqq$ 90 mmHg diastolic after 20 weeks of gestation in an otherwise normotensive woman and proteinuria, consisting of $\geqq$ 300 mg/24-hour urine collection or end-organ dysfunction [1, 2]. For those women already hypertensive prior to pregnancy, superimposed PE can be diagnosed as the worsening of hypertension; it may also present with parallel end-organ damage that is superimposed on preexisting end-organ damage [2].

Prevention of PE is limited. Women at high risk, such as those with a history of PE or those with preexisting chronic hypertension, pregestational diabetes, or twin pregnancy are

recommended to be prescribed daily low-dose aspirin (ASA) early in pregnancy which, in a meta-analysis of 30,000 pregnancies, showed a reduction in both incidence and morbidity of PE [2, 4, 5]. Management of PE once it develops is similarly limited. For women with severe PE, antihypertensive therapy or corticosteroid therapy may be indicated [2]. If those with severe PE reach a gestational age of $\geqq$ 34 weeks, delivery is indicated [1, 2].

Resolution of symptoms occurs with delivery of the placenta [1]. PE may develop in the absence of a fetus as long as placental tissue is present [6]. Inadequate placental perfusion, apoptosis, and an increase in oxidative stress is heavily implicated in the pathophysiology of the disorder [7-11]. The progressive, multisystem vascular dysfunction that the maternal circulation exhibits through worsening PE has been related previously to placental secretions [8, 12, 13].

b. Lipid Abnormalities in Preeclampsia and Aspirin

Initial trials of low-dose aspirin to prevent the development of PE were based on the finding that the placentas of PE women exhibited an increased production of thromboxane and decreased production of prostaglandin I_2 (PGI_2, prostacyclin) [14-17]. Low-dose aspirin can selectively inhibit thromboxane without affecting prostacyclin because thromboxane and prostacyclin are produced by different cells [16, 18]. In the maternal circulation, low-dose aspirin inhibits thromboxane produced by platelets but not prostacyclin produced by endothelial cells [16, 19]. In the placenta, low-dose aspirin inhibits thromboxane produced by the trophoblast cells on the maternal side of the placenta, but only 30% of aspirin crosses the placenta, so prostacyclin produced by the placental endothelial cells on the fetal side are not affected [16, 19]. Oxidative stress in the placenta causes the formation of lipid peroxides [8, 10, 11]. Omega-3-

polyunsaturated fatty acids (PUFAs) are decreased in PE due to their susceptibility to oxidation [20, 21]. Additionally, oxidative stress causes the formation of isoprostanes, which are known to be elevated in PE [11].

c. UO1 Grant

This project operates under the funding and goals of a larger UO1 grant sponsored by the National Institutes of Health's Human Placenta Project. For the UO1 grant, a goal was established to identify a non-invasive way to determine placental function through the identification of a unique profile of bioactive lipids implicit in PE. Identification of lipid biomarkers in maternal blood and urine will be correlated with placental production as measured in this project. Longitudinal collection of blood and urine specimens will be correlated with placental lipids from the same women to determine which maternal lipids originate from the placenta. Clinical applicability will be achieved when a unique lipid "fingerprint" of PE is discerned and plasma or urine specimens can be used to predict the development of PE.

B. Select Lipid Biomarkers and Mediators of Inflammation

a. Polyunsaturated Fatty Acids (PUFAs) and Select Metabolites

i. Eicosapentaenoic Acid (EPA)

EPA, an omega-3 fatty acid, is a obtained in humans from dietary sources such as fish oils and algae [22]. EPA is a constituent of phospholipids in animal tissues. EPA is a precursor of the series-3 prostaglandins (e.g. prostaglandin I_3, thromboxane A_3, prostaglandin E_3, and prostaglandin $F_{3\alpha}$) and resolvins, which themselves are anti-inflammatory. EPA's downstream pathways compete with arachidonic acid (AA) [23].

ii. Docosahexaenoic Acid (DHA)

DHA, an omega-3 fatty acid, is a PUFA that is sourced from fish oils, algae, and other animal phospholipids [22]. It is also a product of alpha-linolenic acid metabolism [22]. DHA inhibits cyclooxygenase enzymes. DHA, through the actions of lipoxygenases, can form anti-inflammatory resolvins and protectins [23, 24].

iii. Resolvins D_1 and D_2

Resolvins D_1 and D_2 are derived from DHA through endogenous lipoxygenases or through cyclooxygenase-2's (COX-2) action as a lipoxygenase in the presence of higher dosages of aspirin, with differences in stereochemistry of the respective products [24, 25]. Resolvins in general exhibit potent anti-inflammatory and immunoregulatory properties [25]. The E series of resolvins, which was not quantified in this project, are generated by similar mechanisms acting on EPA rather than DHA.

iv. Arachidonic Acid (AA)

AA, an omega-6 PUFA**,** is generally the most abundant polyunsaturated component of phospholipid membranes and acts in regulation of apoptosis [26]. It is sourced by dietary intake of the meat of animals and is a metabolite of linoleic acid through dihomo-gamma-linolenic acid (DHGLA) [27]. Free AA, acting as precursor to other eicosanoids, is released through the actions of phospholipase A_2 [24]. AA is the precursor to series-2 prostaglandins (e.g. prostaglandin E_2, prostaglandin I_2, and prostaglandin $F_{2\alpha}$) and thromboxanes (e.g. thromboxane A_2, thromboxane B_2). AA is also the precursor to leukotrienes, lipoxins, hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (EETs), and dihydroxyeicosatrienoic acids (DHETs). An overview of AA's metabolic pathway is shown in Figure 1 [26].

v. Dihomo-Gamma-Linolenic Acid (DHGLA)

DHGLA, an omega-6 PUFA, is derived from linoleic acid and is a precursor to AA. DHGLA is also a precursor to series-1 prostaglandins (e.g. prostaglandin E_1, prostaglandin $F_{1\alpha}$) and

thromboxanes [27]. These eicosanoid metabolites all demonstrate anti-inflammatory effects [28]. Similarities in downstream pathways cause DHGLA to compete with AA for both cyclooxygenase (COX) and lipoxygenases [28].

b. Prostanoids

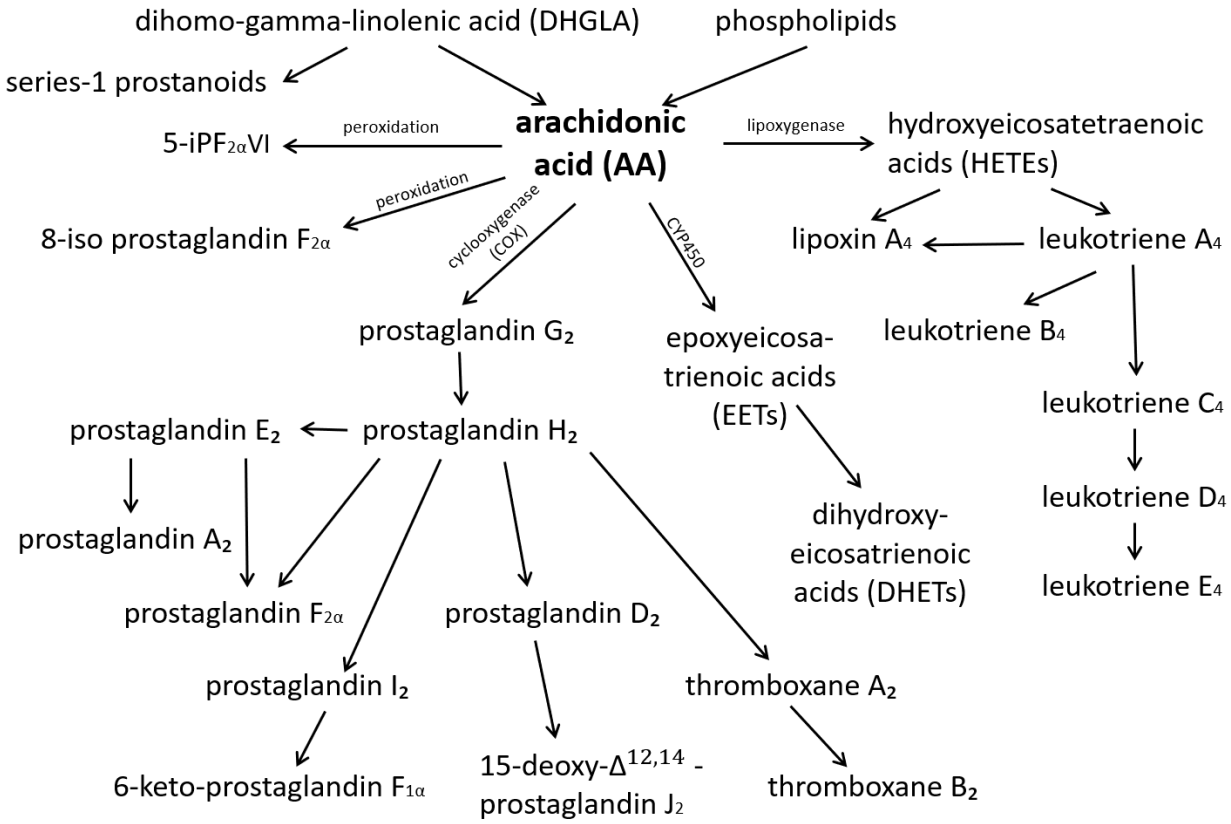


Figure 1. Schematic biosynthetic pathways of eicosanoid metabolism, following arachidonic acid and its series-2 metabolites.

i. 6-keto Prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$)

6-keto $PGF_{1\alpha}$ is the stable metabolite of prostaglandin I_2's (PGI_2, prostacyclin) non-enzymatic hydrolysis *in vivo* (Figure 1) [29]. Prostacyclin, produced by cyclooxygenase (COX) acts as a vasodilator and inhibits platelet aggregation, and can be compared in contrast to the

actions of thromboxane A_2 (TXA_2), which is a vasoconstrictor and stimulator of platelet aggregation [29]. Prostacyclin levels are not decreased by low-dose aspirin therapy because endothelial cells which primarily produce prostacyclin have nucleic and can regenerate COX, whereas platelets, which primarily produce thromboxane, are anuclear and cannot regenerate COX [13-15, 19].

ii. 8-iso Prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$)

8-iso $PGF_{2\alpha}$ is one of the stable products of non-enzymatic peroxidation of AA which can induce vasoconstriction and platelet activation [30]. Due to its generation by peroxidation rather than a COX-mediated pathway, it is a marker of oxidative stress [30].

iii. Prostaglandins E_1 and E_2 (PGE_1, PGE_2)

PGE_1 and PGE_2 are derived from DHGLA and AA respectively through the actions of COX (AA pathway shown in Figure 1) [31]. PGE_1 acts to suppress inflammation while PGE_2, a vasodilator along with other functions, is relevant to this project as it increases uterine tone and can be given therapeutically to induce labor [31].

iv. Prostaglandins $F_{2\alpha}$, D_2, and A_2 ($PGF_{2\alpha}$, PGD_2, PGA_2)

$PGF_{2\alpha}$, PGD_2, and PGA_2 are all pro-inflammatory eicosanoids and are similarly derived from free AA being acted on by COX to form PGG_2, which is metabolized through a peroxidase into the unstable PGH_2 (Figure 1) [29, 32]. PGD_2, PGE_2, and $PGF_{2\alpha}$ are derived by various respective enzymes from PGH_2 (Figure 1) [29]. PGA_2 and $PGF_{2\alpha}$ are derived from dehydration of PGE_2 [29].

v. 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2 (15d-PGJ_2)

15d-PGJ_2 is the dehydration product of PGD_2, and acts as a net anti-inflammatory regulator at the nucleus through induction of apoptotic cell death in activated, inflammatory macrophages [33].

vi. Thromboxane B_2 (TXB_2)

TXB_2 is the stable, inert metabolite of thromboxane A_2 (TXA_2) [34]. TXA_2 shares the common precursor PGH_2 with PGD_2, PGE_2, and $PGF_{2\alpha}$ and is susceptible to inhibition by the effects of low-dose aspirin on cyclooxygenase-1 (COX-1) in platelets and trophoblast cells (Figure 1)[14-16, 19, 29]. TXA_2 acts as a vasoconstrictor and promotes platelet aggregation. It has been shown to be elevated 7-fold in PE [17, 34].

vii. 5-i$PF_{2\alpha}$VI

5-i$PF_{2\alpha}$VI, an isoprostane, is, similarly to 8-iso $PGF_{2\alpha}$, a non-enzymatic peroxidation product of AA used as an indicator of oxidative stress [35].

c. Lipoxygenase and Epoxidation Products of Arachidonic Acid

i. Hydroxyeicosatetraenoic Acids (HETEs) 5-, 12-, 15-, and 20-

5-HETE, 12-HETE, and 15-HETE are all derived from the actions of their respective arachidonate lipoxygenase enzymes (Figure 1) [36]. 5-HETE is a precursor to leukotrienes and

lipoxins (Figure 1) [36]. 12-HETE has varied effects, including promoting chemotaxis, vasoconstriction, and the production of other prostanoids while inhibiting platelet aggregation in opposition to TXA_2 [36]. 15-HETE's production creates 12-HETE as a minor product as well as leukotriene A_4 (LTA_4) [36]. 15-HETE has an inflammatory action, promoting platelet aggregation [36].

20-HETE is an AA metabolite derived from a cytochrome P450 catalyzed reaction [37]. It is a pro-inflammatory, pro-hypertensive eicosanoid which promotes vasoconstriction and can act directly on the TXA_2 receptor [37].

ii. Leukotrienes D_4, C_4, E_4, and B_4 (LTD_4, LTC_4, LTE_4, LTB_4)

The lipoxygenase mediated production of 5-HETE from AA allows the production of LTA_4 (Figure 1) [38]. LTA_4 then acts as a precursor to either LTB_4 or LTC_4, which may then be converted stepwise into LTD_4 and then LTE_4 (Figure 1) [38]. Leukotrienes are pro-inflammatory mediators implicated in asthma and anaphylaxis: LTB_4 is a potent promoter of chemotaxis while LTC_4, LTD_4, and LTE_4 constrict vascular and pulmonary smooth muscle [38].

iii. Lipoxin A_4

Lipoxins are eicosanoids derived from AA and, similarly to resolvins, involved in the resolution phase of inflammation [39]. Biosynthesis can proceed through a common route with 15-HETE or through a lipoxygenase enzyme acting on LTA_4 [39].

iv. Epoxyeicosatrienoic Acids (8,9-EET, 14,15-EET) and Dihydroxyeicosatrienoic Acids (8,9-DHET, 11,12-DHET, 14,15-DHET)

The EET family is formed from the cytochrome P450 epoxidation of AA, and exhibits anti-hypertensive, anti-thrombotic effects and promotes vasodilation [40, 41]. The DHET family are short-lived metabolic products of epoxide hydrolases acting on EETs and are also vasodilators, though may exhibit pro-inflammatory actions [41, 42].

d. Sphingolipids

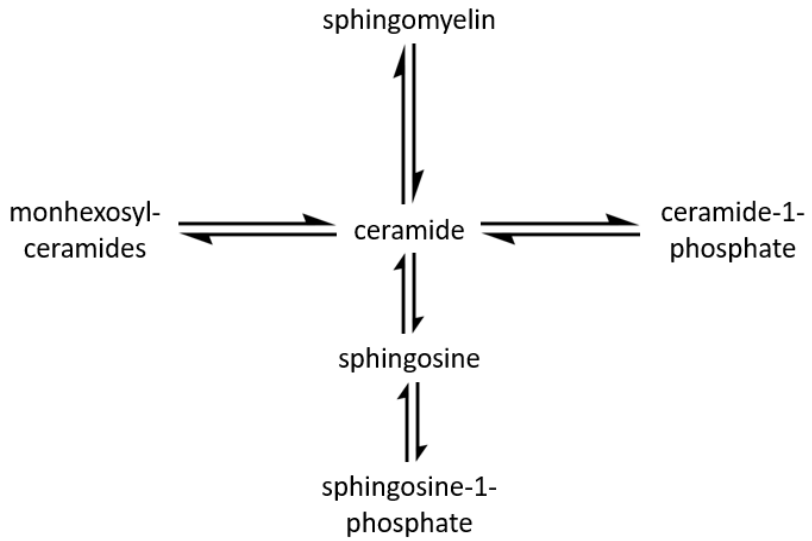


Figure 2. Schematic biosynthetic pathways of sphingolipid interconversion.

i. Ceramides

Ceramides may either be synthesized *de novo* in nearly all cells, created by the enzymatic hydrolysis of sphingomyelin, such as during apoptosis, or generated through catabolism of more complex sphingolipids (Figure 2) [43]. Free bioactive ceramides have been

found to both induce and be present within apoptotic and autophagic cells and are implicated in inflammatory disorders [43, 44].

ii. Monohexosylceramides

Monohexosylceramides, such as galactosylceramide and glucosylceramide, are synthesized by a specific glycosyl-transferase catalyzed reaction of ceramide (Figure 2) [45]. Monohexosylceramides function in the plasma membrane, conferring specificity in binding to a wide range of proteins [45].

iii. Sphingomyelin

Sphingomyelins, the most abundant sphingolipid, are synthesized from phosphatidylcholine and ceramide, as catalyzed by sphingomyelin synthase. Diacylglycerol, a second messenger, is a byproduct of this synthesis [46]. The functions of sphingomyelins are generally involved with acting as a reservoir for sphingolipid precursors and the regulation of cell membrane lipid rafts [46].

iv. Ceramide-1-Phosphates (C1Ps)

C1Ps exhibit anti-apoptotic properties and stimulate the release of free AA from phospholipids through phospholipase A2, promoting eicosanoid biosynthesis [47, 48]. They are formed from ceramide by the specific enzyme, ceramide kinase, and may be readily converted back to ceramide by a phosphatase (Figure 2).

v. Sphingosine-1-Phosphates (S1Ps)

S1Ps are synthesized by the stepwise deacylation of ceramide into sphingosine and subsequent phosphorylation of sphingosine into S1Ps [44]. S1Ps act as anti-apoptotic sphingolipids with many actions antagonistic to other pro-apoptotic sphingolipids [44].

C. Purpose of Investigation

Specific Aim 1: To determine placental lipid production with regards to inflammatory mediators in PE women.

Hypothesis: The placentas of women diagnosed with PE will favor the production of pro-inflammatory lipids.

Specific Aim 2: To determine the differences in placental lipid production between early onset and late onset PE.

Hypothesis: The placentas of women who experienced early onset PE will produce more pro-inflammatory lipids than the placentas of those who experienced late onset PE.

Specific Aim 3: To determine the overall lipid profiles (fingerprints) produced by placentas of women with PE, late onset PE, and early onset PE.

Hypothesis: There is a distinct lipid profile which characterizes each of these groups.

Specific Aim 4: To determine the changes in placental lipid production in response to aspirin therapy.

Hypothesis: Placental lipid production in patients receiving aspirin therapy will demonstrate a reduction in thromboxane, an increase in prostacyclin, and possible other effects on cyclooxygenase metabolites.

D. Rationale for Studies

Investigation was done on placental tissue as it is the suspected organ which mediates the pathology of PE [6-8, 12, 13]. The pathophysiological state that a PE placenta manifests is predicted to produce lipids not only for a localized inflammatory response but also circulating lipids that mediate the maternal disease state. The chorionic villus tissue that was used in the explant culture is bathed in maternal blood *in vivo* and as such its metabolic products enter the maternal circulation [7]. Of the various mediators of inflammation such as vasoactive amines, cytokines or the complement system, lipids were selected because PE is known to be associated with an imbalance of placental eicosanoids [2, 13]. The development of high throughput, wide range lipid analysis provides a large pool of lipids for which a fingerprint may be identified to predict and identify a placental lipid profile consistent with the PE disease state.

Chapter 2: Methods and Materials

A.Recruitment and Categorization

Pregnant women for this study were recruited from VCU's low-risk and high-risk pregnancy clinics. Patients were briefed and signed a consent form early in their pregnancy. These women were also used within the larger UO1 grant for the longitudinal analysis of blood and urine throughout pregnancy. This study, within the larger UO1 grant, was approved by the Office of Research Subjects Protection of Virginia Commonwealth University.

High-risk clinic patients are, by clinic procedure, referred there for a history of: previous PE, chronic hypertension, diabetes, preterm births, intrauterine growth restriction, cervical insufficiency, drug use, or cardiovascular disease. High-risk patients may also be referred following genetic screening or referral from the low-risk clinic as pregnancy problems develop. Following delivery, the placenta was collected and transported securely to the laboratory for processing. Gestational ages, final pregnancy status, and any fetal, placental, or known pathological anomalies were recorded.

Categorization of patients was performed to identify those that were not diagnosed with PE, a normal pregnancy (NP), against those who were diagnosed with PE. Diagnosis for PE was done by physicians in the clinic per ACOG guidelines (see Chapter 1: Introduction, Section A. General Background). Within the PE category, further division was made between those who, prior to delivery, reached a gestational age of greater than or equal to 37.0 weeks and were deemed late-onset preeclampsia (LOPE) and those delivered before reaching a gestational age of 37.0 weeks, deemed early-onset preeclampsia (EOPE). No consideration was made for whether the placenta was delivered through Caesarean section or through vaginal birth.

Most PE patients were recruited from the high-risk clinic. Patients at risk for PE are placed on low-dose aspirin therapy according to ACOG guidelines [2]. Therefore, most of the EOPE patients were most likely on low-dose aspirin therapy. One of the Obstetrics and Gynecology residents is documenting and tracking the use of aspirin, but that information is not available as of the writing of this thesis.

B.Placental Tissue Preparation

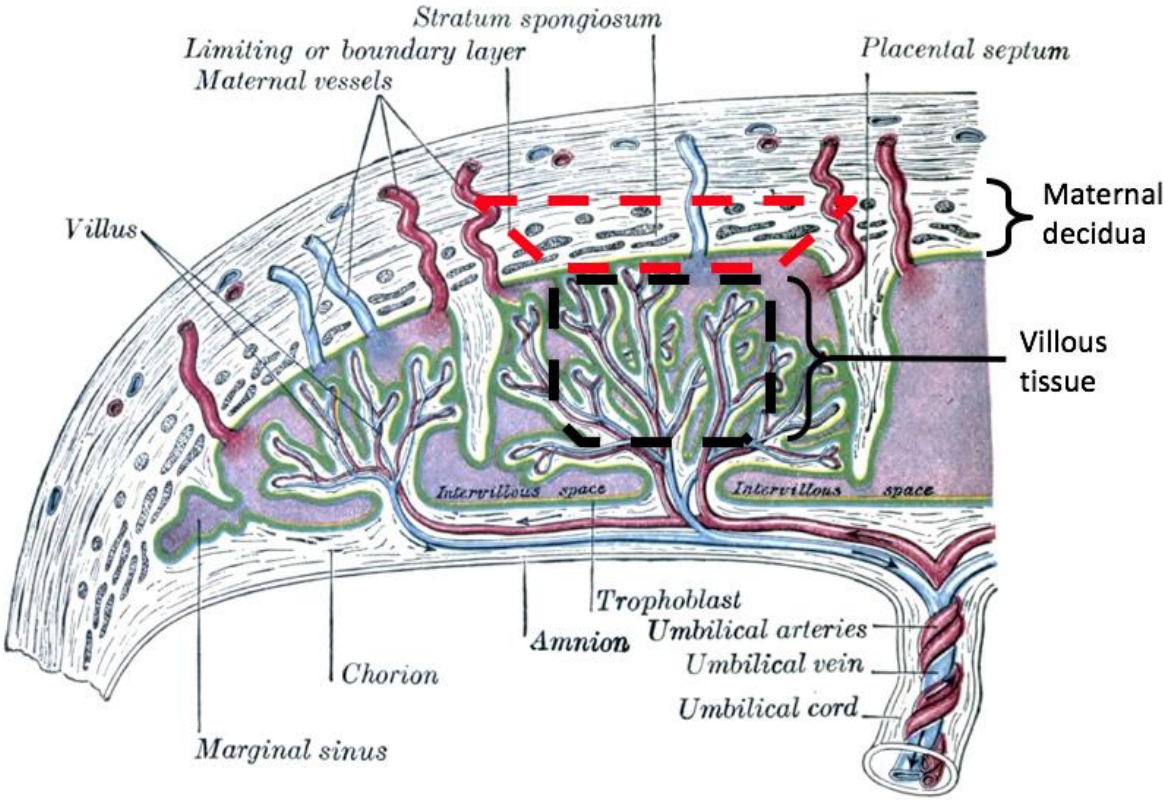


Figure 3. Schematic of placental circulation and anatomy. Overlying maternal decidua was first removed, indicated by red dotted box. Myometrium, above red box, is not delivered with the placenta. An excised region where chorionic villous tissue could be obtained for culture is indicated with the black dotted box; multiple, representative samples of this region were taken from each placenta. Image from the public domain [49].

Explant culture of placenta tissue was performed within 2 hours of delivery to ensure tissue viability. Excision of representative samples of placental villous tissue from different cotyledons was performed after removal of the overlying layer of maternal decidual tissue (Figure 3). For placentas with pending pathology studies, a single placental cotyledon was sampled to obtain villous tissue. Selection of sample sites from each placenta was performed to avoid regions with calcification, excess connective tissue, and visible blood vessels in the tissue. To avoid cross-contamination, excision of the underlying villous tissue was performed with

separate instruments from those used on the decidual surface of the placenta, which may be contaminated with vaginal or fecal bacteria when the placenta was delivered vaginally.

Excised villous tissue was minced with sterile surgical instruments in a sterile petri dish (Thermo Scientific™, Cat. # 168381) suspended in a sterile phosphate buffered saline (PBS) (Gibco™, Cat. # 10010023) bath to increase surface area of the tissue. It was then rinsed repeatedly with PBS to remove blood and strained through a 4”x4” section of sterile gauze (Fisherbrand™, Cat. # 22-415-469). Approximately 500 mg of solid tissue was placed in culture wells of a six well cell culture plate (Corning®, Cat. # 3506) along with 5 mL of Media 199 (Gibco™, Cat. # 11150059) containing a mixture of 100 units/mL each of the antibiotics penicillin and streptomycin (Gibco™, Cat. # 15140122). Media 199 was used with neither serum nor phenol red. Culture wells with media and tissue were incubated for 48 hours in a Heracell™ 150i incubator (Thermo Scientific™, Cat. # 51026280) at 37.0 °C and 5.0% CO_2.

Following incubation, the cultured media was aspirated and placed into a 15 mL sterile conical tube (USAScientific™, Cat. # 1475-1611) and centrifuged (Thermo Scientific™, Cat. # 75391762) at 4000 xg for 10 minutes. The media was removed, aliquoted, and stored at -80 °C in cryogenic storage vials (Fisherbrand™, Cat. # 12567502) for later lipidomic analysis. The remaining tissue pellet was saved and frozen at -80 °C in the 15 mL conical tubes for later protein quantification.

C. Placental Protein Quantification and Normalization

For protein quantification, the frozen tissue pellet was removed from the -80 °C freezer and placed into a polystyrene weighing boat (Fisherbrand™, Cat. # 08732116). Frozen tissue pellet was weighed to determine mass after culturing. Liquid nitrogen was added onto the tissue pellet in the weighing boat to flash freeze the tissue for ease of homogenization. The tissue was quickly transferred into a 75 mm round bottom polystyrene test tube (Fisherbrand™, Cat. # 14-959-16A). Tissue was homogenized in 5 mL PBS using a tissue homogenizer (PRO Scientific™, Cat. # 01-01200). Homogenized tissue in suspension was centrifuged at 4000 xg for 20 minutes. Supernatant was then quantified for protein using BCA colorimetric kit (Pierce™, Cat. # 23225) against BSA standards (Pierce™, Cat. # 23209) with 25 μL samples in a 96 well plate (Corning®, Cat. # 3590). Plate reading and linear regression was performed using MARS Data Analysis Software and FLUOstar OPTIMA Microplate Reader (BMG LABTECH). Results of protein quantification were presented by the software as mcg protein per mL of sample, which was multiplied by 5 mL to get total protein for the tissue. Lipidomic results were then divided by total protein. An example is presented in Equation 1. Final units used for analysis and graphing were pg lipid per mcg protein for eicosanoids and pmol lipid per mg protein for sphingolipids.

Equation 1. Sample calculation for normalization of an eicosanoid lipid results from media to the respective protein content of tissue from the same explant culture well. Lipidomics results are divided by protein quantification results.

$$39.76\,\frac{ng\ TXB_2}{mL\ media} \div\ 3456\,\frac{mcg\ protein}{mL\ sample} \times\ 1000\,\frac{pg}{ng} =\ 11.50\ \frac{pg\ TXB_2}{mcg\ protein}$$

D. Placental Media Lipidomic Analysis

Lipidomic analysis of a total of 75 eicosanoids and sphingolipids was performed through the Virginia Commonwealth University's Lipidomics/Metabolomics Shared Resource (VLMC) and through a similar facility located at the University of South Florida. Electrospray ionization mass spectrometry (ESI-MS) for eicosanoids was performed using a QTRAP 5500 LC-MS/MS System (AB Sciex) following concentration of samples via a solid phase extraction column followed by the quantitative, targeted analysis of 30 analytes using deuterated standards. Mass spectrometry for sphingolipids was performed following extraction of the samples using a modified Bligh-Dyer method followed by the quantitative, targeted analysis of each of the 45 analytes.

Lipid results were delivered in ng/mL for eicosanoids and pmol/mL for sphingolipids, normalized against the volumes of media used for testing. Lipidomic analysis was also done on plain, uncultured media to provide a control comparison to each lipid analyzed. Three sphingolipids were excluded as they did not read in a detectable range: the C16 DH variants of monohexosylceramides and sphingomyelin and sphingosine-1-phosphate variant C18:1 So1P.

Prior to statistical analysis, normalization against the mass of protein per mass of placenta tissue in each incubated well was performed on the respective media's lipidomic result.

E.Statistical Analysis

Analysis was done using statistical software (PRISM 6, GraphPad, San Diego, CA). Outlier analysis for each lipid analyzed was performed using the Robust regression and Outlier removal (ROUT) (Q=0.5%) methodology in PRISM. Significant differences between categories were evaluated by separate unpaired t-tests as compared to the NP category (Table 2). ANOVA was not used because the PE subcategories (EOPE and LOPE) were not independent from the PE category. A p-value of $< .05$ was considered statistically significant. For each of the three PE categories, percent changes from NP for each lipid were also calculated for graphical display.

Chapter 3: Results

A. Data Characteristics

Among all 74 placentas, gestational ages at birth ranged from 24.4 – 41.5 weeks, with an average of 37.5 weeks. Of the 74 placentas, 21 were collected from patients from VCU's low-risk pregnancy clinic. The remaining 53 placentas were collected from patients from VCU's high-risk pregnancy clinic. Patient demographics such as age, ethnicity, obstetric history, medical history, and the original reason for referral to high-risk clinic is currently being investigated for each patient. That information is not available as of the writing of this thesis.

Protein quantification yielded protein concentrations ranging from 1895 mcg to 5060 mcg, with an average of 3664 mcg of protein per tissue pellet (i.e. per 500 mg whole, wet tissue).

Table 1. Characteristics of patient categories. Values displayed are mean ± standard deviation.

Variable	**NP**	**PE**	**LOPE**	**EOPE**
Sample Size	50	24	12	12
From High-Risk Clinic	34	19	9	10
From Low-Risk Clinic	16	5	3	2
Gestational Age (weeks)	38.5 ± 1.7	35.5 ± 3.9	38.4 ± 1.2	32.9 ± 3.7

B. Eicosanoid Results

a. Polyunsaturated Fatty Acids (PUFAs) and Select Metabolites

i. Eicosapentaenoic Acid (EPA)

EPA was significantly increased for EOPE compared to NP (Figure 4: a).

ii. Docosahexaenoic Acid (DHA)

DHA was significantly increased for PE and EOPE compared to NP (Figure 4: b).

iii. Resolvins D_1 and D_2

Absolute lipidomic results obtained were low for resolvin D_1. Resolvin D_1 had an average value of 0.21 ng/mL media, within an order of magnitude of the negative controls. There were no significant differences for resolvins for PE, LOPE or EOPE as compared to NP (Figure 4: c, d).

iv. Arachidonic Acid (AA)

There were no significant differences AA for PE, LOPE or EOPE as compared to NP (Figure 4: e).

v. Dihomo-Gamma-Linolenic Acid (DHGLA)

DHGLA was significantly increased for PE, LOPE or EOPE compared to NP (Figure 4: f).

b. Prostanoids

i. 6-keto Prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$)

6-keto $PGF_{1\alpha}$ was significantly increased for PE, LOPE, and EOPE compared to NP (Figure 4: g).

ii. 8-iso Prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$)

8-iso $PGF_{2\alpha}$ was significantly increased for PE and EOPE compared to NP (Figure 4: h).

iii. Prostaglandins E_1 and E_2 (PGE_1, PGE_2)

PGE_1 and PGE_2 were significantly decreased for EOPE compared to NP (Figure 4: i, j).

iv. Prostaglandins $F_{2\alpha}$, D_2, and A_2 ($PGF_{2\alpha}$, PGD_2, PGA_2)

Absolute lipidomic results obtained were low for PGA_2, with an average of 2.91 ng/mL media, within an order of magnitude of the negative controls. There were no significant differences for $PGF_{2\alpha}$, PGD_2, and PGA_2 for PE, LOPE, or EOPE as compared to NP (Figure 4: k, l, m).

v. 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2)

Absolute lipidomic results obtained were low for the metabolite of prostaglandin J_2, with an average of 1.20 ng/mL media, within an order of magnitude of the negative controls. There were no significant differences for 15dPGJ_2 for PE, LOPE or EOPE as compared to NP (Figure 4: n).

vi. Thromboxane B_2 (TXB_2)

There were no significant differences for TXB_2 for PE, LOPE or EOPE as compared to NP (Figure 4: o).

vii. 5-i$PF_{2\alpha}$VI

There were no significant differences for 5-i$PF_{2\alpha}$VI for PE, LOPE or EOPE as compared to NP (Figure 4: p).

c. Lipoxygenase and Epoxidation Products of Arachidonic Acid

i. Hydroxyeicosatetraenoic Acids (HETEs) 5-, 12-, 15-, and 20-

HETEs were consistently and significantly increased in EOPE compared to NP (Figure 4: q, r, s, t). All but 12-HETE were also increased in PE compared to NP (Figure 4: q, s, t).

ii. Leukotrienes D_4, C_4, E_4, and B_4 (LTD_4, LTC_4, LTE_4, LTB_4)

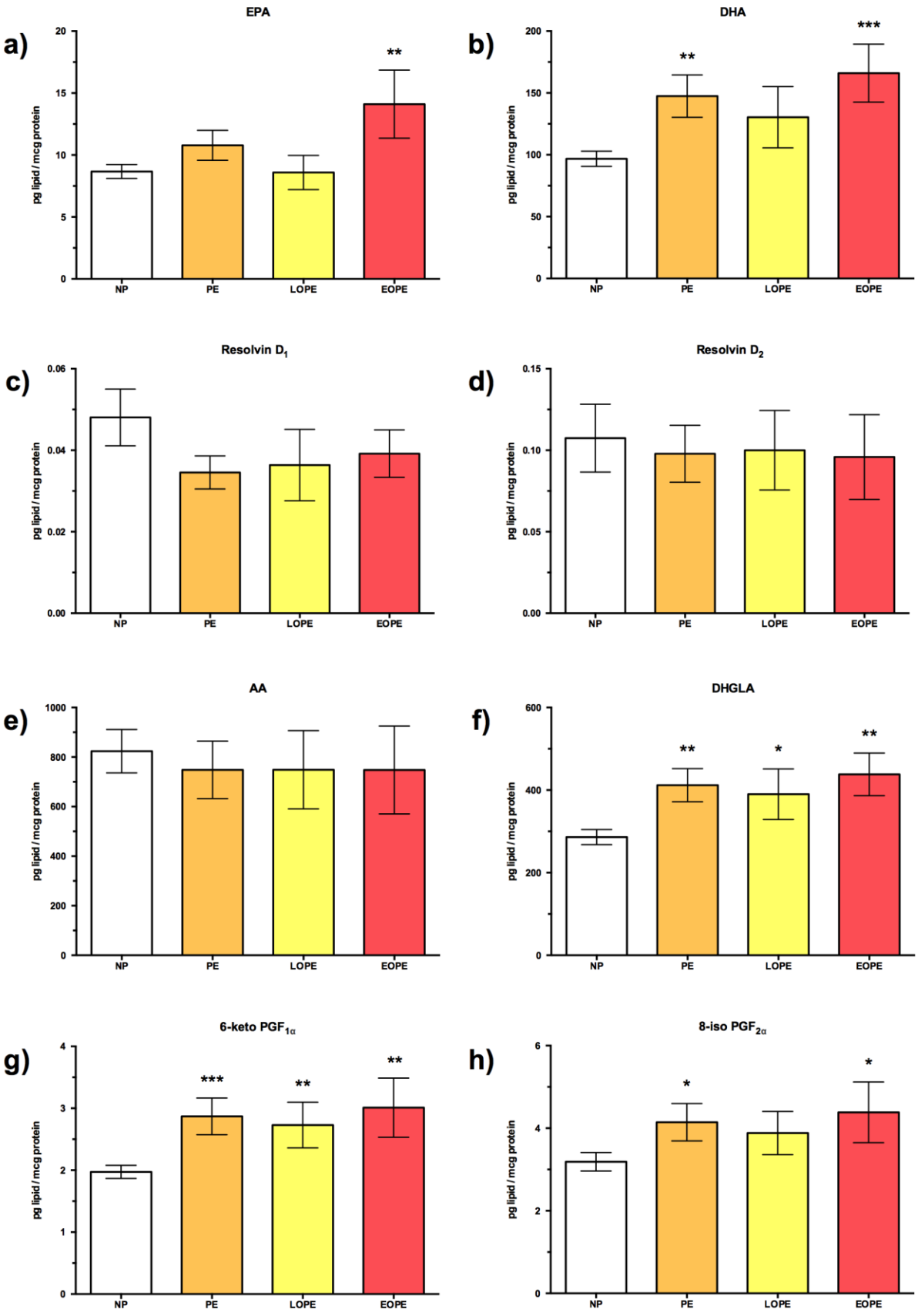
Lipidomic results were low for leukotrienes. Respective averages of 0.14, 0.23, 0.15, and 2.90 ng/mL of media, each within an order of magnitude of the negative controls, were measured for LTD_4, LTC_4, LTE_4, and LTB_4. There were numerous null readings (i.e. undetectable) within mass spectrometry runs for leukotrienes. There were no significant differences for the leukotrienes for PE, LOPE or EOPE as compared to NP (Figure 4: u, v, w, x).

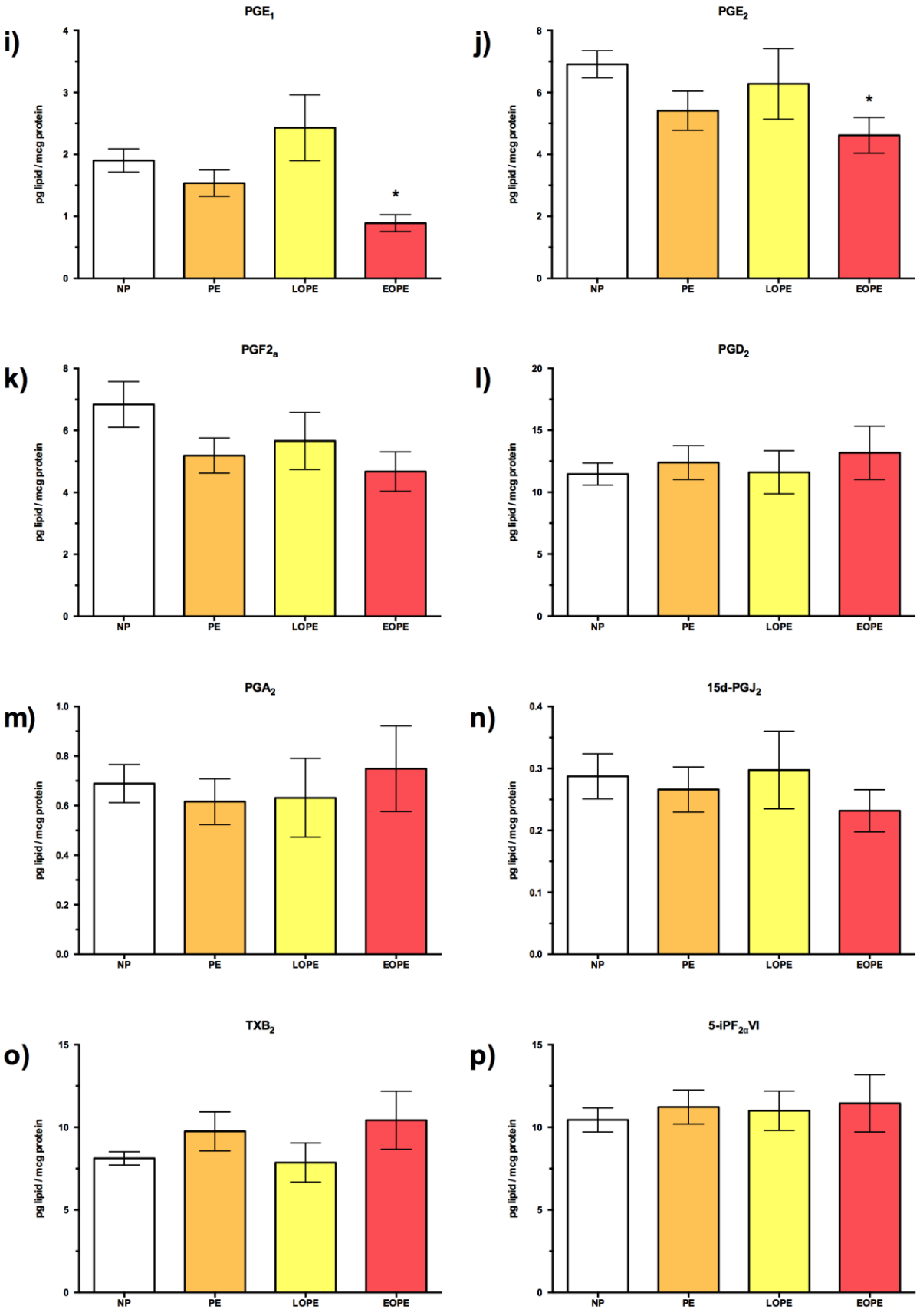
iii. Lipoxin A_4

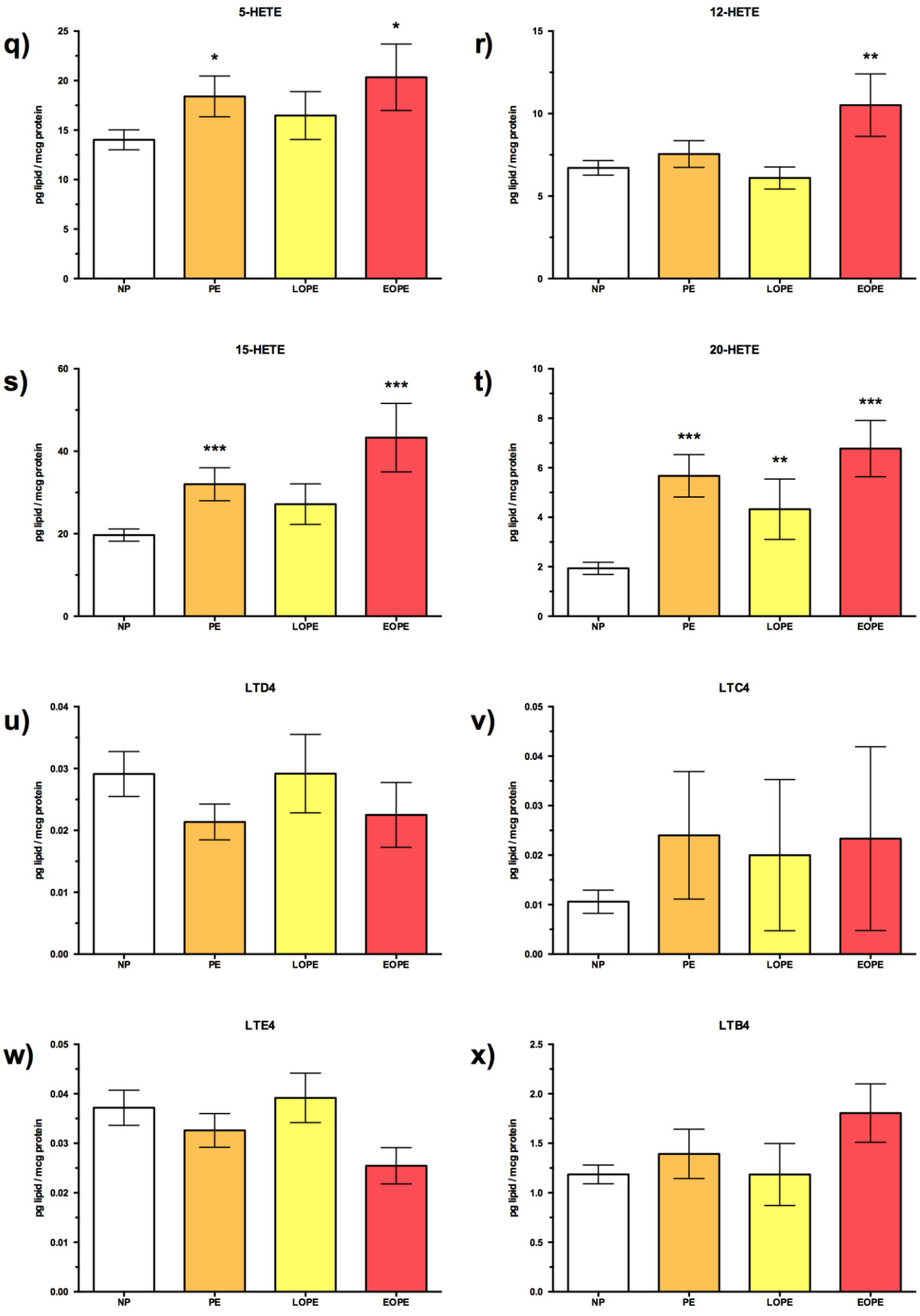
There were no significant differences for Lipoxin A_4 for PE, LOPE or EOPE as compared to NP (Figure 4: y).

iv. Epoxyeicosatrienoic Acids (8-9-EET, 14,15-EET) and Dihydroxyeicosatrienoic Acids (8,9-DHET, 11,12-DHET, 14,15-DHET)

11,12-DHET and 14,15-DHET were significantly increased in all categories (PE, LOPE, and EOPE) compared to NP (Figure 4: ac, ad). For 8-9-EET, 14,15-EET, and 8,9-DHET there were no significant differences for PE, LOPE or EOPE as compared to NP (Figure 4: z, aa, ab).


a)
EPA
pg lipid / mcg protein
NP
PE
LOPE
EOPE
**
b)
DHA
**
***
c)
Resolvin D1
d)
Resolvin D2
e)
AA
f)
DHGLA
*
g)
6-keto PGF1α
h)
8-iso PGF2α


i)
PGE1
pg lipid / mcg protein
NP
PE
LOPE
EOPE
*
j)
PGE2
pg lipid / mcg protein
NP
PE
LOPE
EOPE
*
k)
PGF2a
pg lipid / mcg protein
NP
PE
LOPE
EOPE
l)
PGD2
pg lipid / mcg protein
NP
PE
LOPE
EOPE
m)
PGA2
pg lipid / mcg protein
NP
PE
LOPE
EOPE
n)
15d-PGJ2
pg lipid / mcg protein
NP
PE
LOPE
EOPE
o)
TXB2
pg lipid / mcg protein
NP
PE
LOPE
EOPE
p)
5-iPF2αVI
pg lipid / mcg protein
NP
PE
LOPE
EOPE


q)
5-HETE
pg lipid / mcg protein
NP
PE
LOPE
EOPE
r)
12-HETE
s)
15-HETE
t)
20-HETE
u)
LTD4
v)
LTC4
w)
LTE4
x)
LTB4

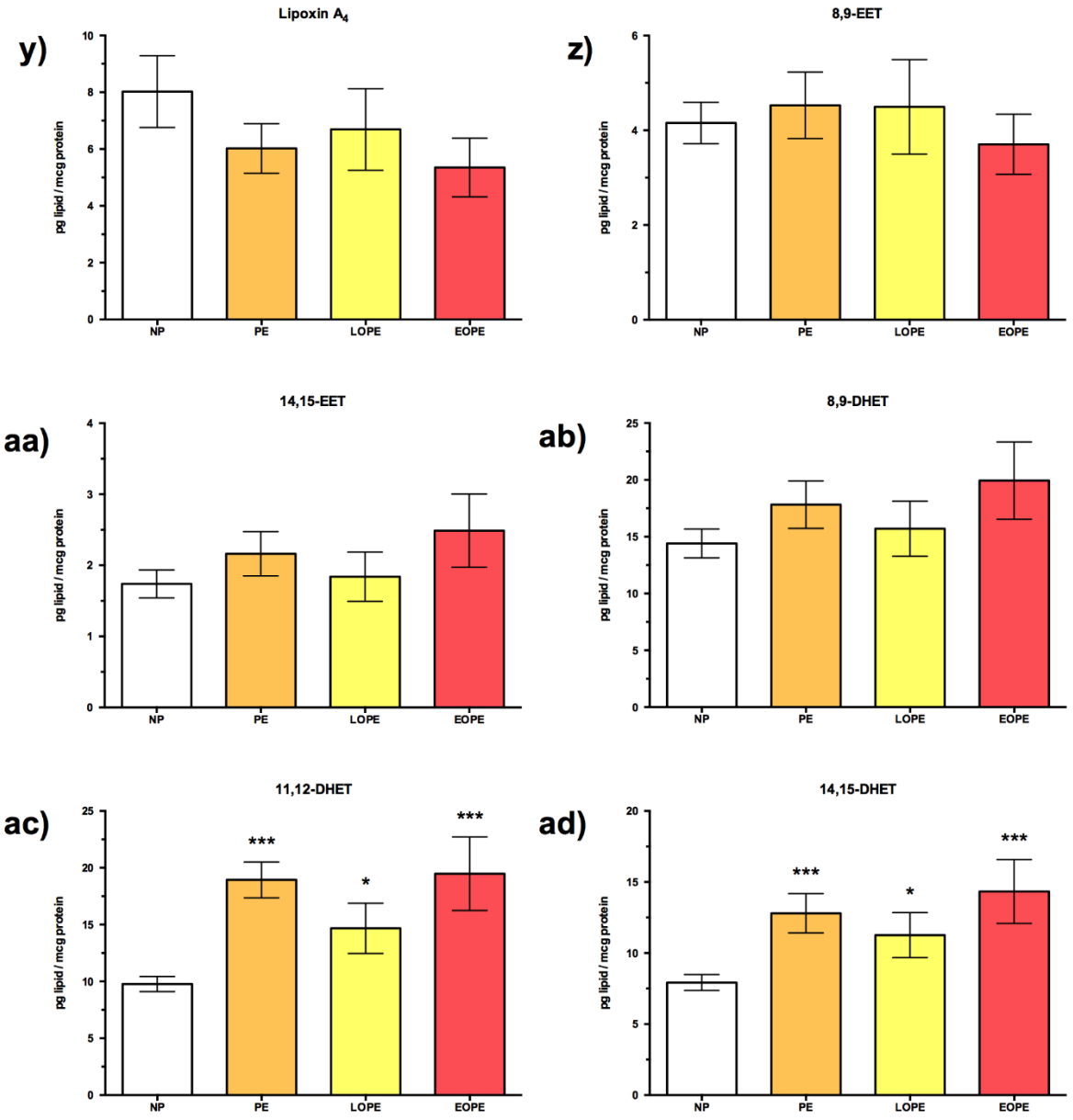


Figure 4. Comparison of average eicosanoid concentrations for patient categories.

Error bars shown are *SEM*. Asterisks signify level of significance between the category labeled below the asterisk as compared to the NP category.
If a p-value is less than .05 it is flagged with one star (*). If a p-value is less than .01 it is flagged with two stars (**). If a p-value is less than .001 it is flagged with three stars (***).

C. Sphingolipid Results

a. Ceramides

Ceramide C14:0 was decreased for PE and LOPE as compared to NP (Figure 5: a). Ceramide C16:0 and C18:0 were increased for EOPE as compared to NP (Figure 5: b, d).

b. Monohexosylceramides

Lipidomic results were low for the C14:0, C18:1, C18:0, C20:0, C26:1, and C26:0 variants of monohexosylceramides, with respective averages of 0.42, 0.34, 0.55, 0.36, 0.68, and 0.13 pmol/mL media, each within an order of magnitude of the negative controls (Figure 5: k, m, n, o, s, t). Monohexosylceramides C24:0 were significantly decreased for PE and EOPE as compared to NP (Figure 5: r). Monohexosylceramides C18:1 were significantly increased for PE, LOPE, and EOPE as compared to NP (Figure 5: m).

c. Sphingomyelins

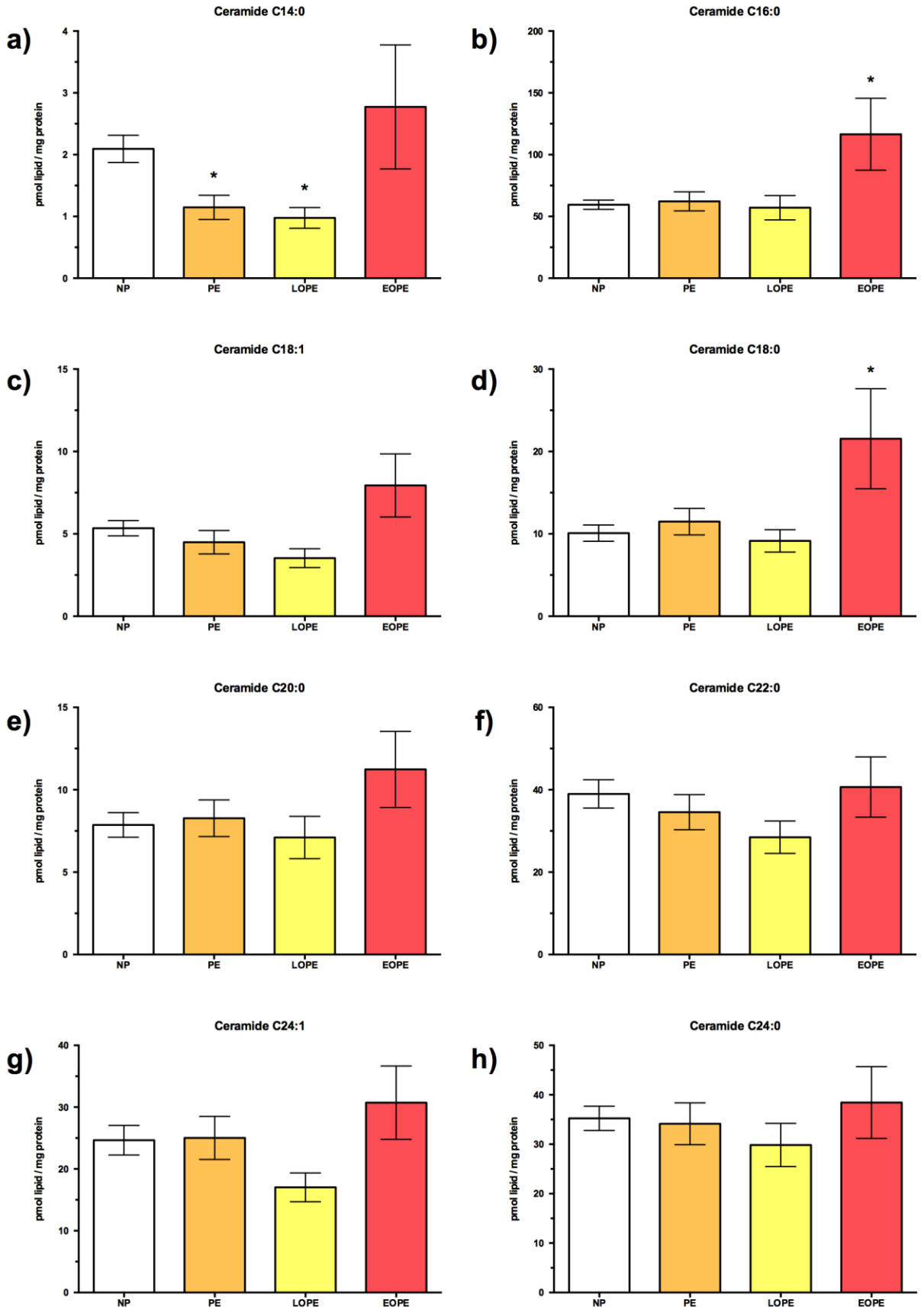
Sphingomyelin C18:0 and C22:0 were increased from NP to EOPE (Figure 5: x, z). The C18:0 variant was also increased from NP to PE (Figure 5: x).

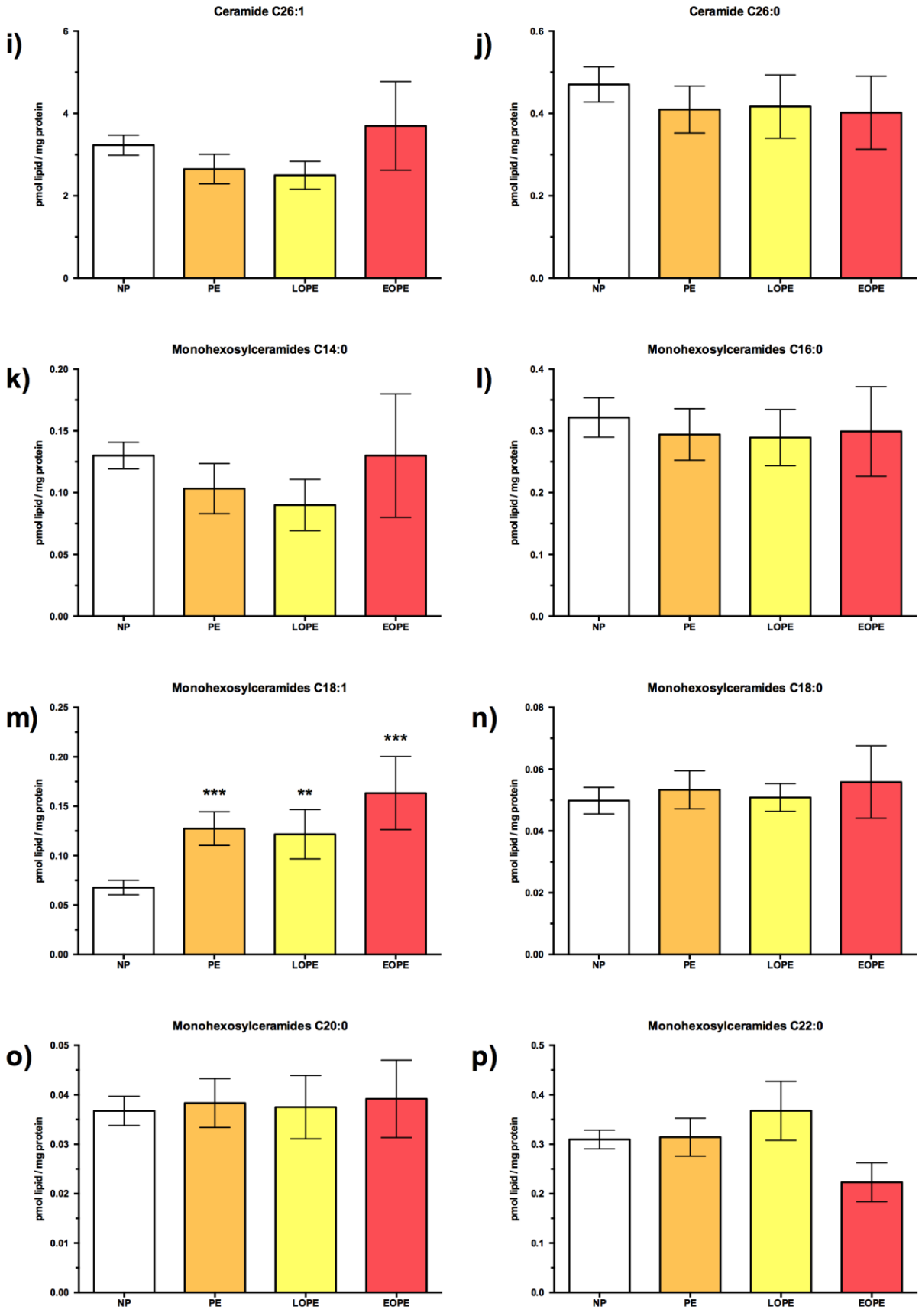
d. Ceramide-1-Phosphates (C1P)

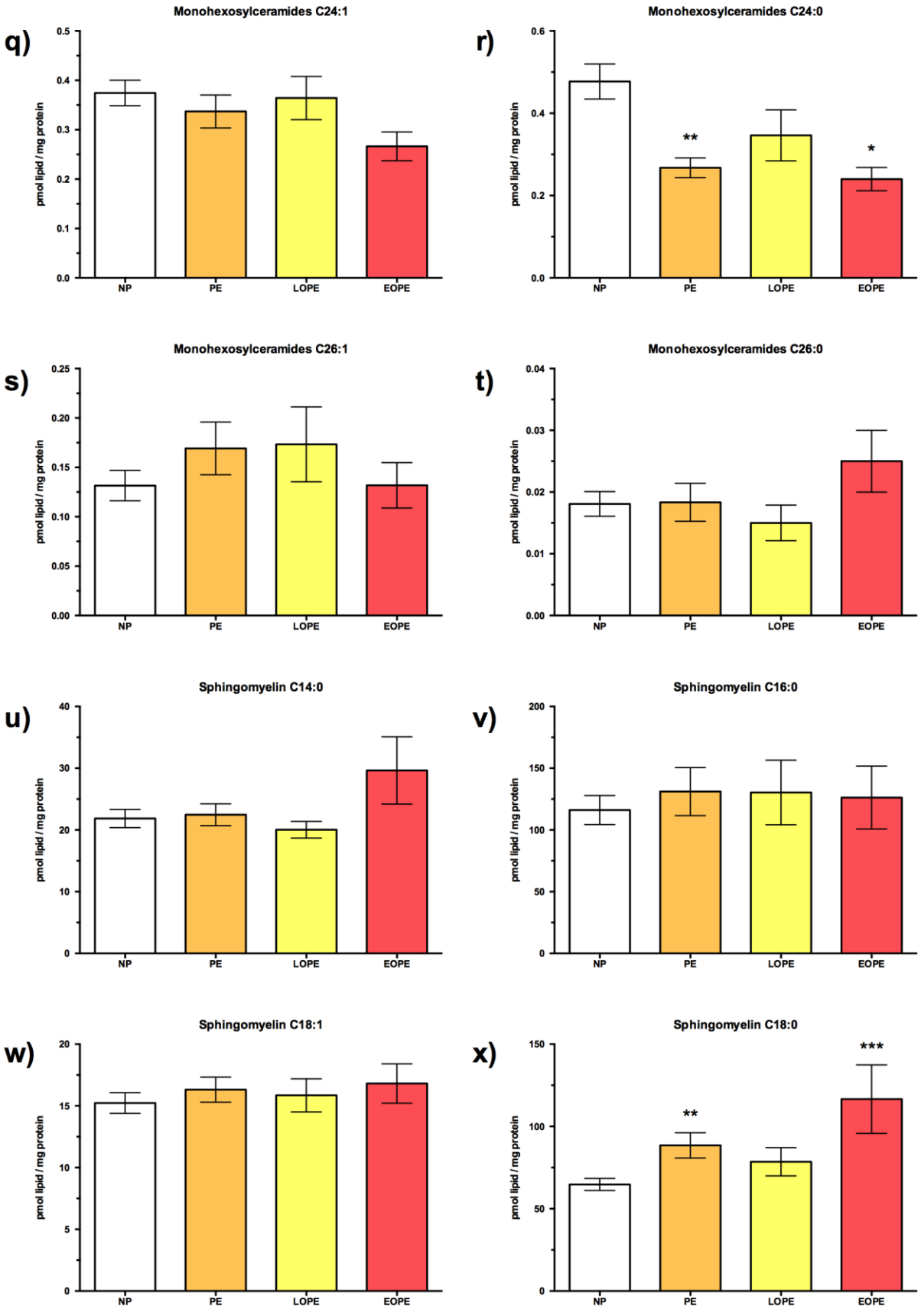
Lipidomic results were low for the C14:0, C18:1, C18:0, C20:0, C22:0, C24:0, C26:1, and C26:1 variants of C1P, with respective averages of 0.19, 0.40, 0.52, 0.14, 0.61, 0.90, 0.05, and 0.09 pmol/mL media, each within an order of magnitude of the negative controls (Figure 5: ae, ag, ah, ai, aj, al, am, an). C1P C14:0 was increased for PE, LOPE, and EOPE compared to NP (Figure 5: ae). C1P C20:0 was increased for EOPE compared to NP (Figure 5: ai). C1P C24:0 was decreased for PE and LOPE as compared to NP (Figure 5: al).

e. Sphingosine-1-Phosphates (S1P)

The two S1P variants both increased from NP to EOPE (Figure 5: ao, ap). The C18:1 S1P variant also increased from NP to PE (Figure 5: ao).


a)
Ceramide C14:0
pmol lipid / mg protein
NP
PE
LOPE
EOPE
b)
Ceramide C16:0
c)
Ceramide C18:1
d)
Ceramide C18:0
e)
Ceramide C20:0
f)
Ceramide C22:0
g)
Ceramide C24:1
h)
Ceramide C24:0


i)
Ceramide C26:1
pmol lipid / mg protein
0
2
4
6
NP
PE
LOPE
EOPE
j)
Ceramide C26:0
pmol lipid / mg protein
0.0
0.2
0.4
0.6
NP
PE
LOPE
EOPE
k)
Monohexosylceramides C14:0
pmol lipid / mg protein
0.00
0.05
0.10
0.15
0.20
NP
PE
LOPE
EOPE
l)
Monohexosylceramides C16:0
pmol lipid / mg protein
0.0
0.1
0.2
0.3
0.4
NP
PE
LOPE
EOPE
m)
Monohexosylceramides C18:1
pmol lipid / mg protein
0.00
0.05
0.10
0.15
0.20
0.25
***
**
***
NP
PE
LOPE
EOPE
n)
Monohexosylceramides C18:0
pmol lipid / mg protein
0.00
0.02
0.04
0.06
0.08
NP
PE
LOPE
EOPE
o)
Monohexosylceramides C20:0
pmol lipid / mg protein
0.00
0.01
0.02
0.03
0.04
0.05
NP
PE
LOPE
EOPE
p)
Monohexosylceramides C22:0
pmol lipid / mg protein
0.0
0.1
0.2
0.3
0.4
0.5
NP
PE
LOPE
EOPE


q)
Monohexosylceramides C24:1
pmol lipid / mg protein
0.0
0.1
0.2
0.3
0.4
0.5
NP
PE
LOPE
EOPE
r)
Monohexosylceramides C24:0
pmol lipid / mg protein
0.0
0.2
0.4
0.6
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NP
PE
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s)
Monohexosylceramides C26:1
pmol lipid / mg protein
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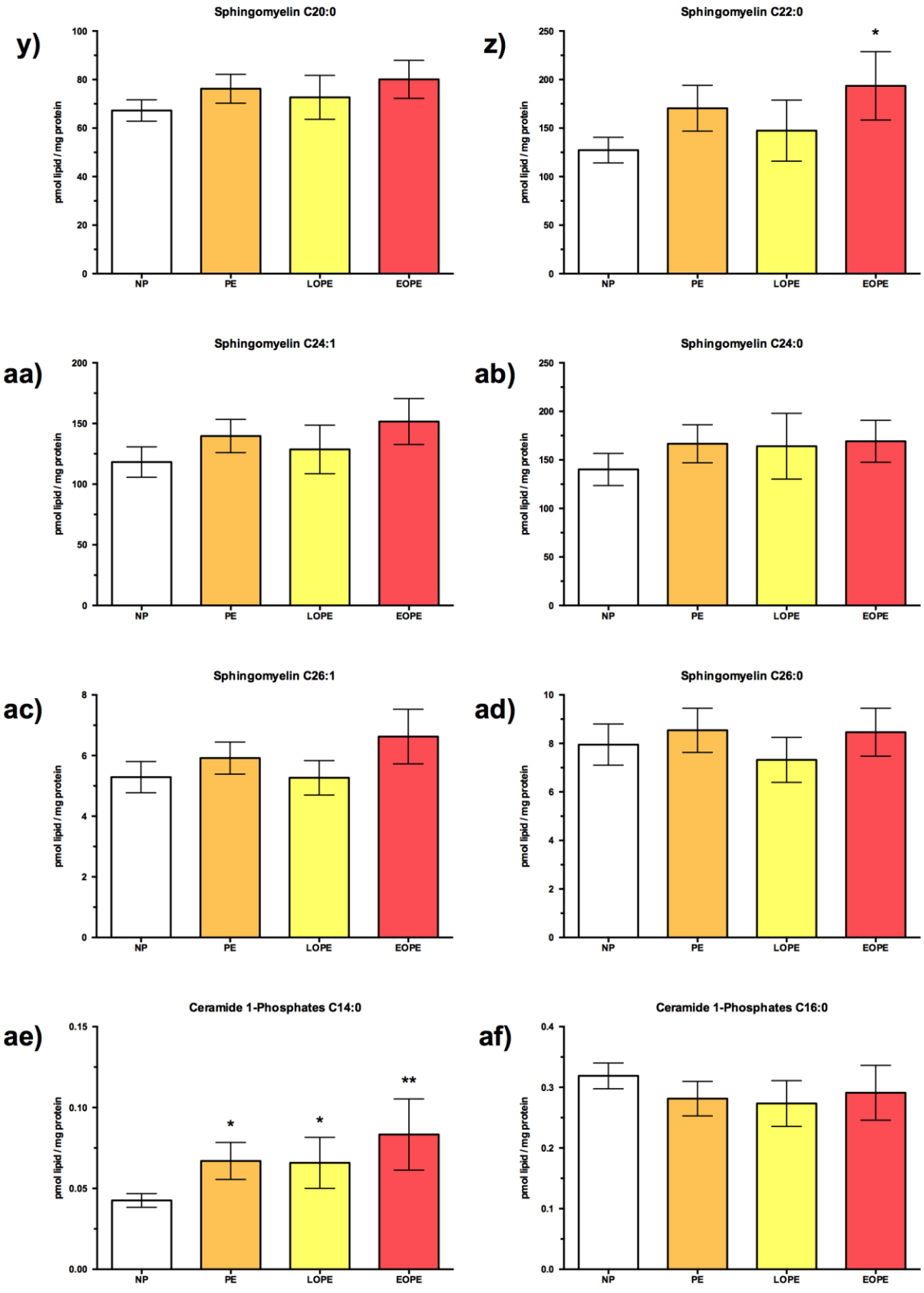
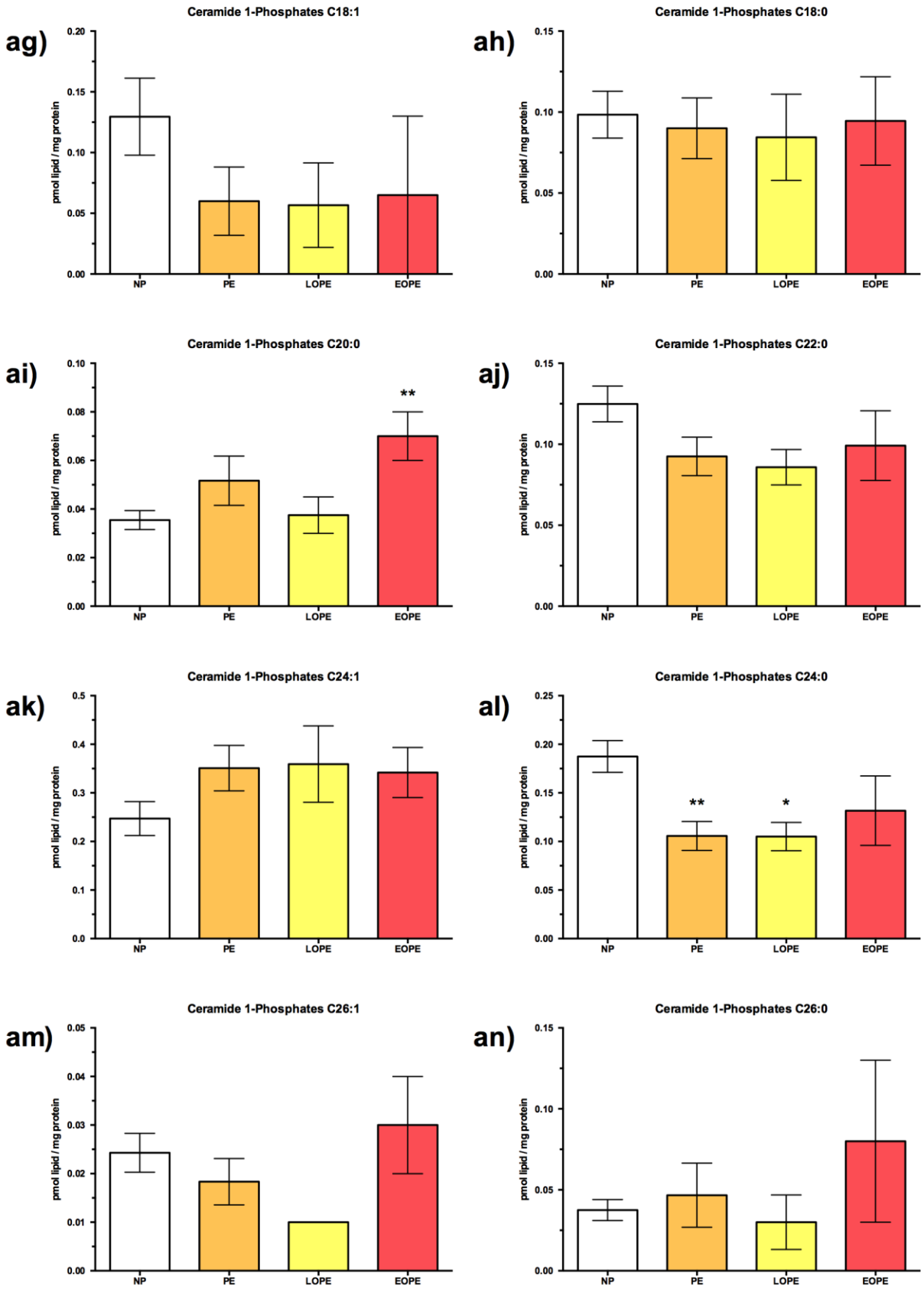
0.05
0.10
0.15
0.20
0.25
NP
PE
LOPE
EOPE
t)
Monohexosylceramides C26:0
pmol lipid / mg protein
0.00
0.01
0.02
0.03
0.04
NP
PE
LOPE
EOPE
u)
Sphingomyelin C14:0
pmol lipid / mg protein
0
10
20
30
40
NP
PE
LOPE
EOPE
v)
Sphingomyelin C16:0
pmol lipid / mg protein
0
50
100
150
200
NP
PE
LOPE
EOPE
w)
Sphingomyelin C18:1
pmol lipid / mg protein
0
5
10
15
20
NP
PE
LOPE
EOPE
x)
Sphingomyelin C18:0
pmol lipid / mg protein
0
50
100
150
**
***
NP
PE
LOPE
EOPE


y)
Sphingomyelin C20:0
pmol lipid / mg protein
NP
PE
LOPE
EOPE
z)
Sphingomyelin C22:0
*
aa)
Sphingomyelin C24:1
ab)
Sphingomyelin C24:0
ac)
Sphingomyelin C26:1
ad)
Sphingomyelin C26:0
ae)
Ceramide 1-Phosphates C14:0
*
*
**
af)
Ceramide 1-Phosphates C16:0


ag)
Ceramide 1-Phosphates C18:1
pmol lipid / mg protein
0.20
0.15
0.10
0.05
0.00
NP
PE
LOPE
EOPE
ah)
Ceramide 1-Phosphates C18:0
pmol lipid / mg protein
0.15
0.10
0.05
0.00
NP
PE
LOPE
EOPE
ai)
Ceramide 1-Phosphates C20:0
pmol lipid / mg protein
0.10
0.08
0.06
0.04
0.02
0.00
**
NP
PE
LOPE
EOPE
aj)
Ceramide 1-Phosphates C22:0
pmol lipid / mg protein
0.15
0.10
0.05
0.00
NP
PE
LOPE
EOPE
ak)
Ceramide 1-Phosphates C24:1
pmol lipid / mg protein
0.5
0.4
0.3
0.2
0.1
0.0
NP
PE
LOPE
EOPE
al)
Ceramide 1-Phosphates C24:0
pmol lipid / mg protein
0.25
0.20
0.15
0.10
0.05
0.00
**
*
NP
PE
LOPE
EOPE
am)
Ceramide 1-Phosphates C26:1
pmol lipid / mg protein
0.05
0.04
0.03
0.02
0.01
0.00
NP
PE
LOPE
EOPE
an)
Ceramide 1-Phosphates C26:0
pmol lipid / mg protein
0.15
0.10
0.05
0.00
NP
PE
LOPE
EOPE

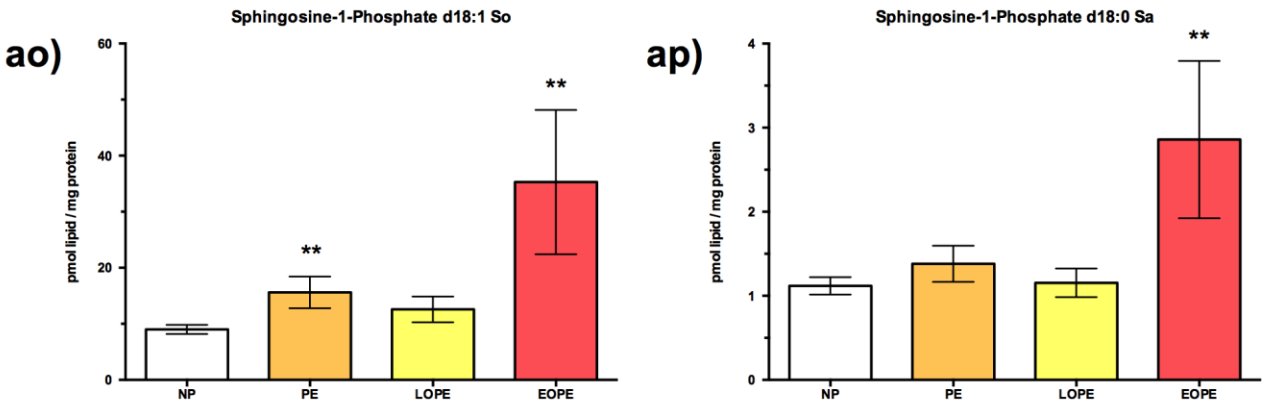


Figure 5. Comparison of average sphingolipid concentrations for patient categories.

Error bars shown are *SEM*. Asterisks signify level of significance between the category labeled below the asterisk as compared to the NP category.
If a p-value is less than .05 it is flagged with one star (*). If a p-value is less than .01 it is flagged with two stars (**). If a p-value is less than .001 it is flagged with three stars (***).

D. Percent Changes for Significantly Altered Lipids

For each of the lipids, using the average lipid concentrations for the PE, LOPE, and EOPE categories, a percent change from that lipid's average concentration for the NP category was calculated. An example is presented in *Equation 2*.

Equation 2. Sample calculation for percent change of TXB_2 for EOPE as compared to NP.

$$\%\ Change = \frac{(TXB_2\ avg.for\ EOPE) - (TXB_2\ avg.for\ NP)}{(TXB_2\ avg.for\ NP)} \times 100$$

$$\frac{9.99\frac{pg\ lipid}{mcg\ protein} - 8.53\frac{pg\ lipid}{mcg\ protein}}{8.53\frac{pg\ lipid}{mcg\ protein}} \times 100\% = 17.11\%$$

To graphically present the changes in lipid productions of placentas in the different categories of PE, lipids which were statistically significant in their differences from NP were selected. For these lipids, radar graphs were generated (Figure 6, Figure 7). A correlation is shown between more dramatic changes in placental lipid productions (i.e. a greater percent change) and the perceived severity of PE, as indicated by the gestational age at delivery (EOPE or LOPE). For the sphingolipids, an average of each category of sphingolipid is shown and only the individual carbon chain lengths which deviate from that category's average are shown. For the sphingolipids interconverted in the sphingomyelin cycle (ceramide, sphingosine-1-

phosphate, and sphingomyelin) a radar graph displaying the relation between the C18:0 variants were generated (Figure 8).

To graphically present the relative increases and decreases each lipid undergoes as a percent change from the NP category to the PE category, lipids were sorted in descending order. Eicosanoids sorted by percent change are all displayed in Figure 9. Sphingolipids which increased from NP to PE are shown in Figure 10, while those that decreased are shown in Figure 11.

Figure 6. For the eicosanoids which demonstrated statistically significant differences, above is a graphical comparison of percent changes between the averages of each category (PE, LOPE, or EOPE) compared to the average of NP (shown as a blue circle at 0% change from itself).

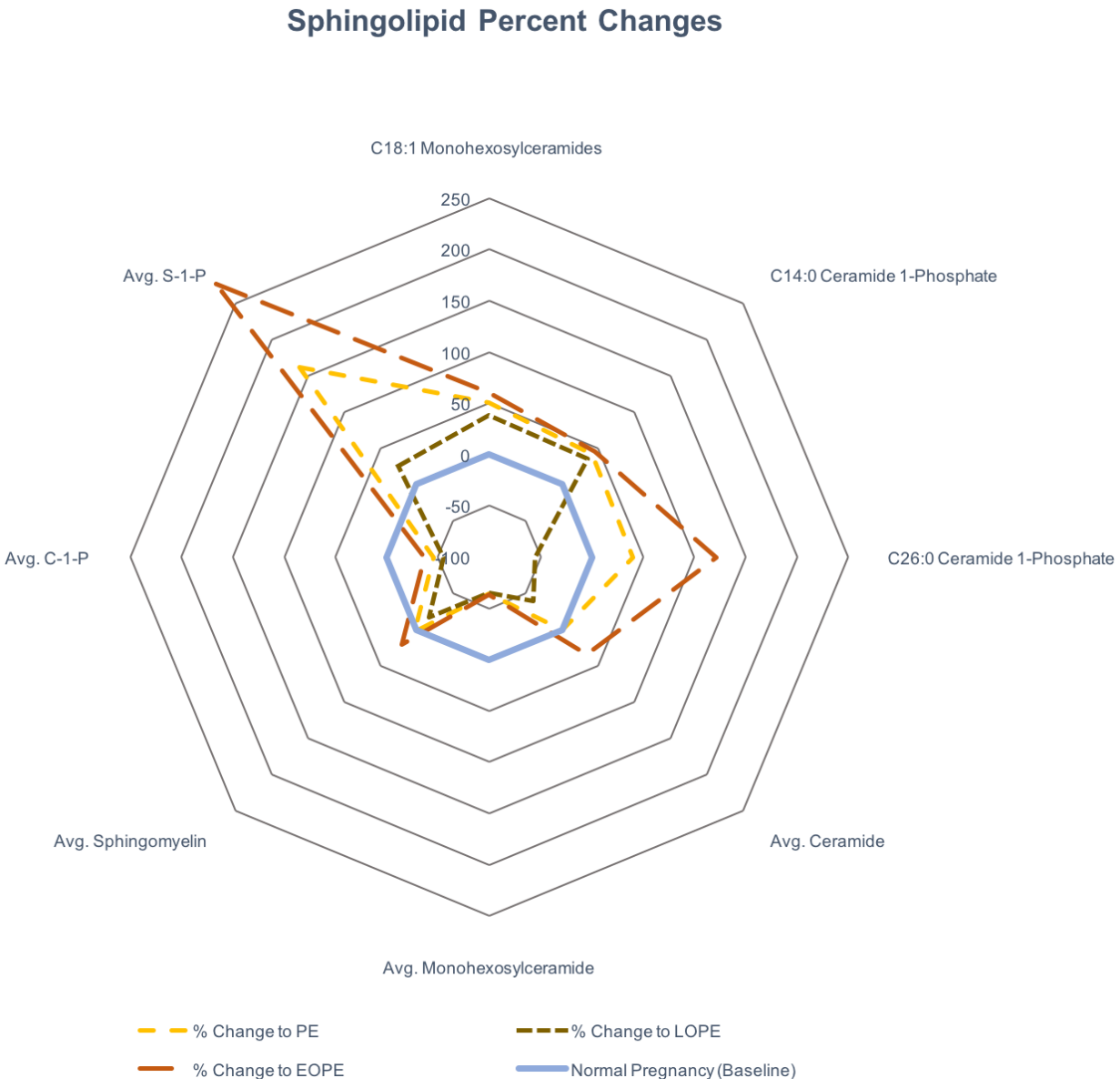


Figure 7. For the sphingolipids which demonstrated statistically significant differences, above is a graphical comparison of percent changes between the averages of each category (PE, LOPE, or EOPE) compared to the average of NP (shown as a blue circle at 0% change from itself). Averages for all variants of each sphingolipid type (ceramide, monohexosylceramide, sphingomyelin, C1P, or S1P) are also shown.

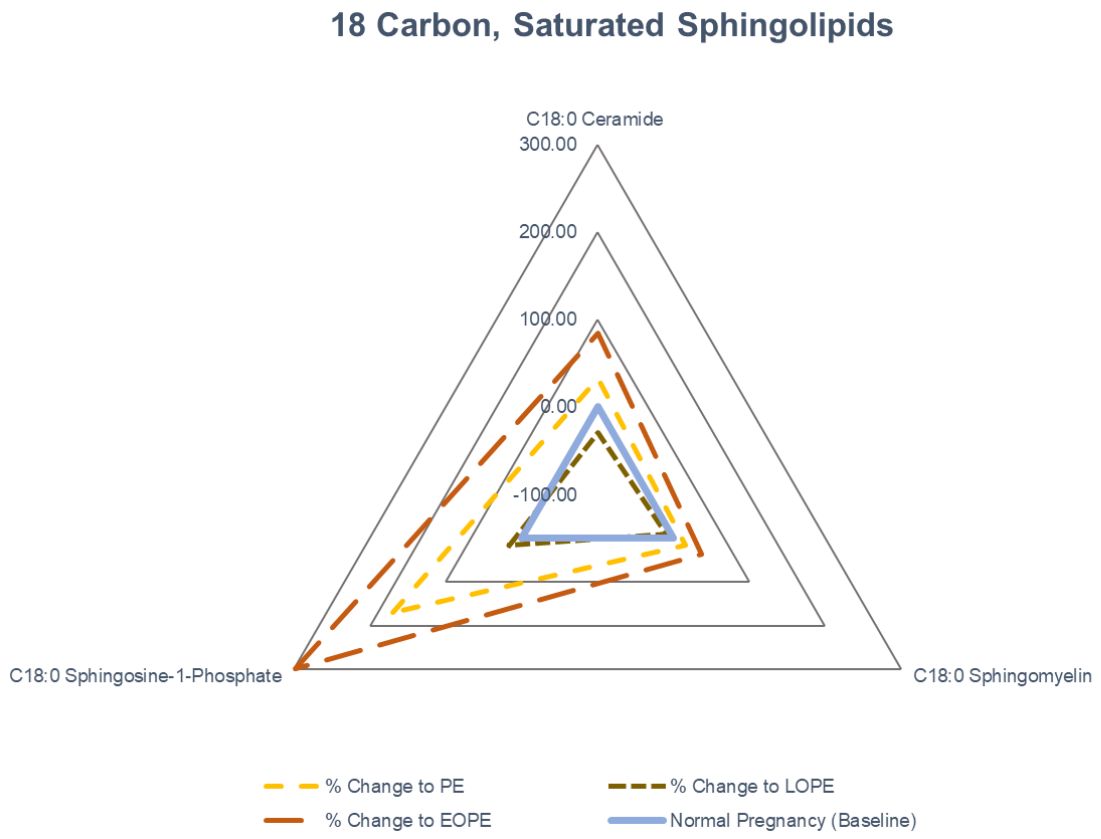


Figure 8. For interconverted C18:0 sphingolipids, above is a graphical comparison of percent changes between the averages of each category (PE, LOPE, or EOPE) compared to the average of NP (shown as a blue triangle at 0% change from itself).

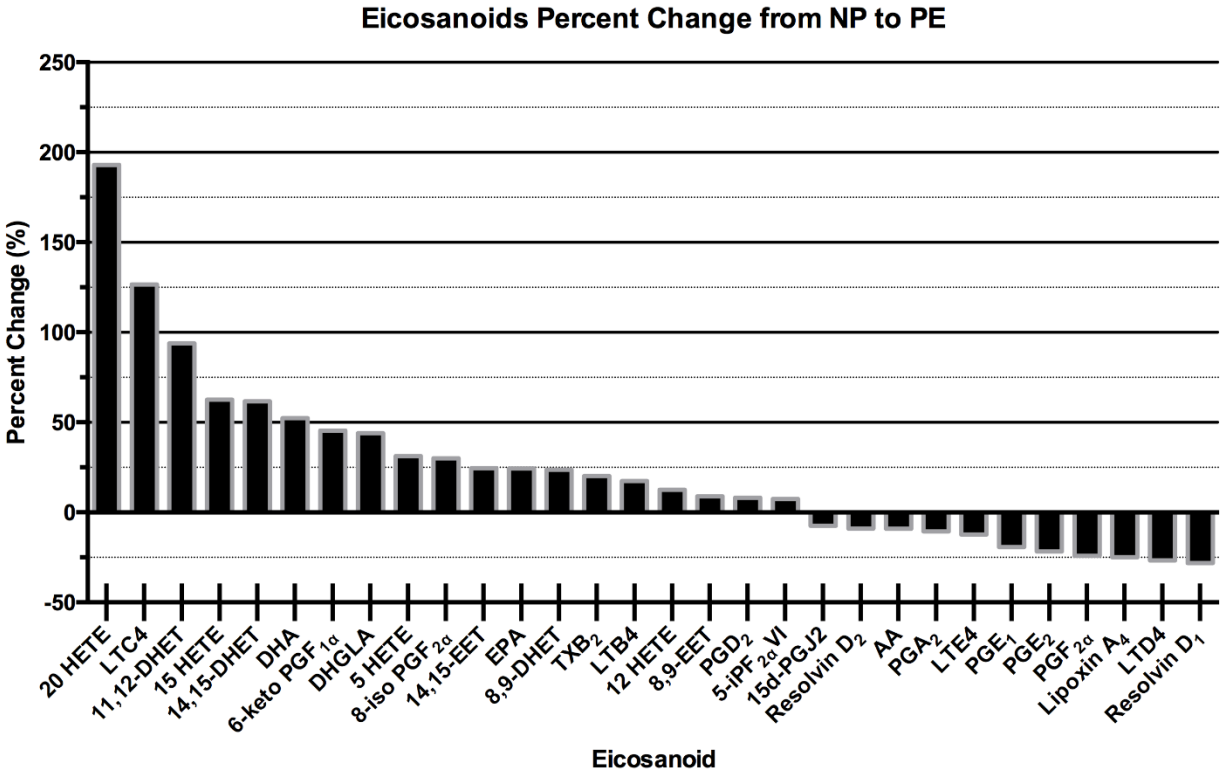


Figure 9. For all analyzed eicosanoids, above is a graphical representation of percent changes between the average lipid concentrations of the PE category compared to the average of the NP category. Lipids sorted in a descending order by their respective percent change.

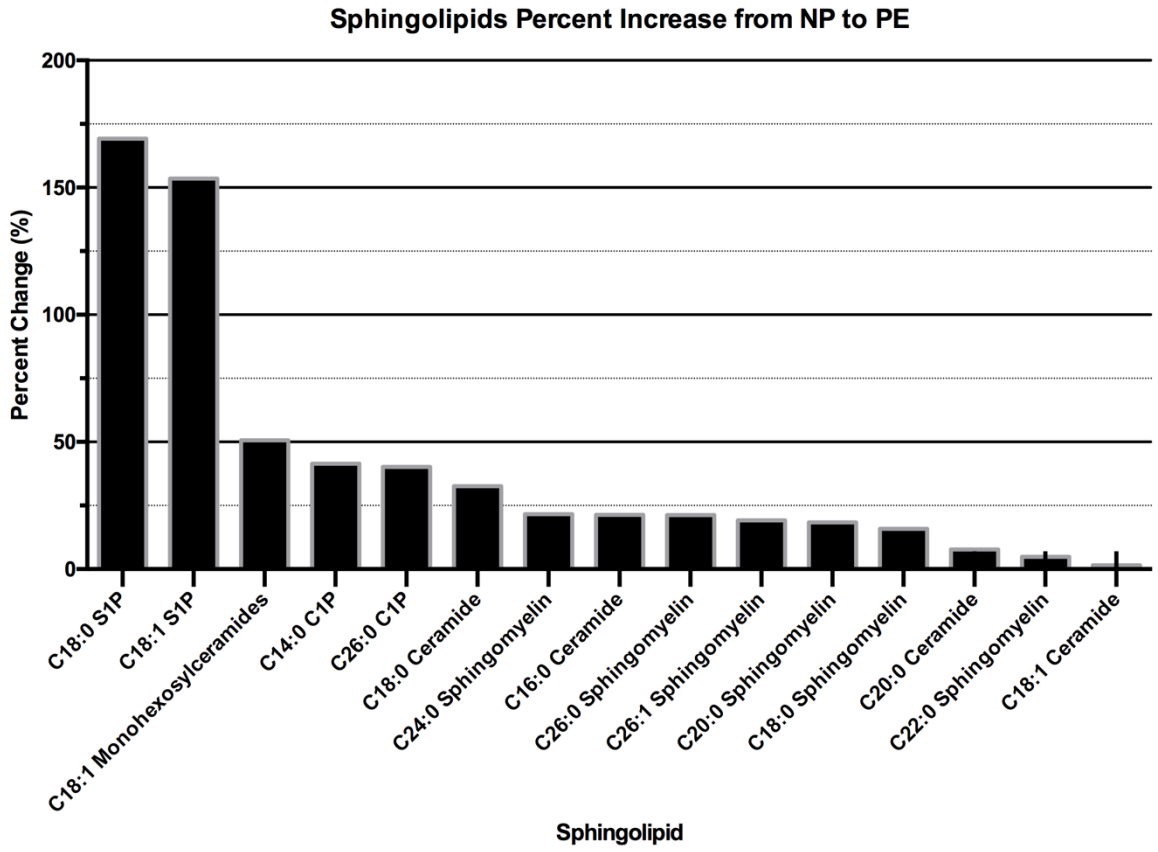


Figure 10. For sphingolipids with a positive percent change, above is a graphical representation of those percent changes. Percent changes shown are between the average lipid concentrations of the PE category compared to the average of the NP category. Lipids sorted in a descending order by their respective percent change.

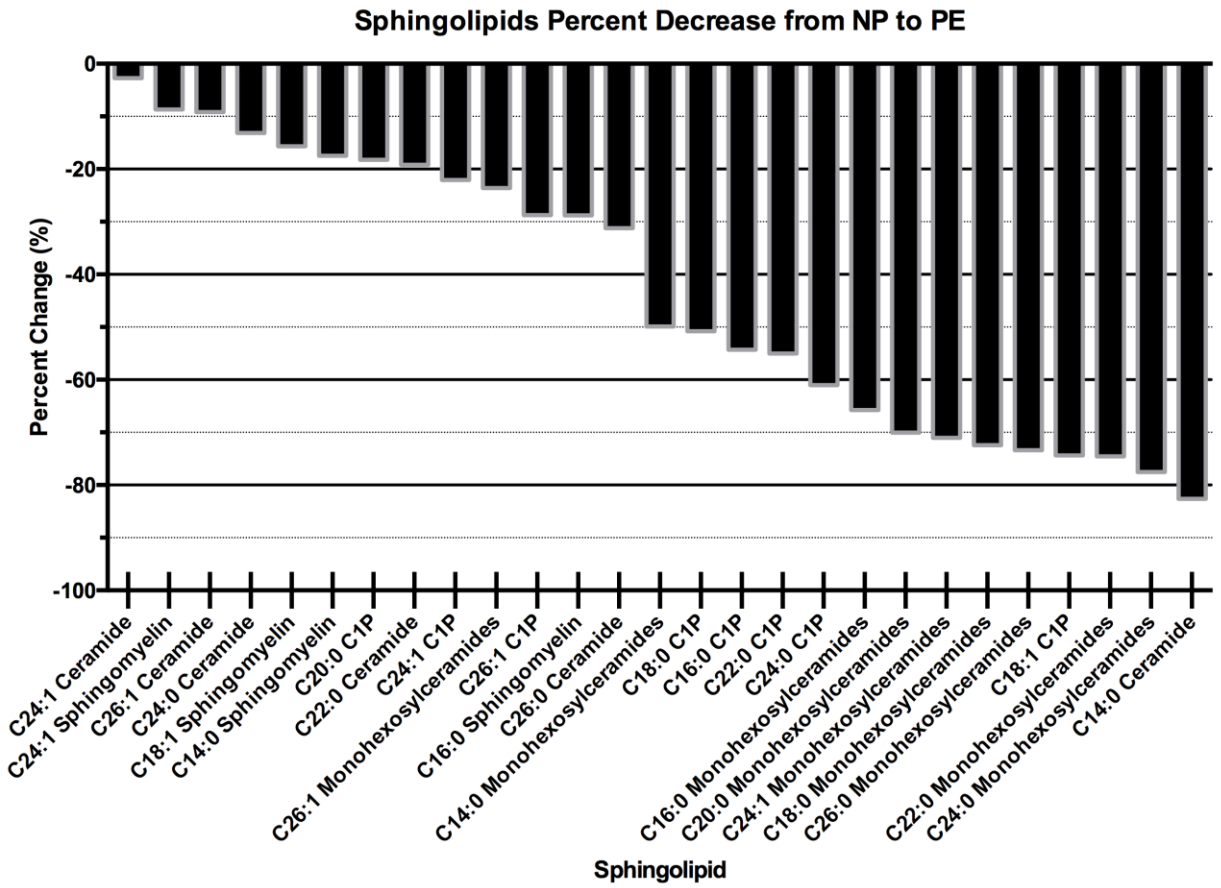


Figure 11. For sphingolipids with a negative percent change, above is a graphical representation of those percent changes. Percent changes shown are between the average lipid concentrations of the PE category compared to the average of the NP category. Lipids sorted in a descending order by their respective percent change.

Chapter 4: Discussion

A. Eicosanoids

The placental eicosanoid results obtained in this study are consistent with the use of low-dose aspirin. Placental thromboxane production was not elevated while prostacyclin was found to be increased. Low-dose aspirin may have been attenuating the oxidative stress that would otherwise decrease prostacyclin synthesis [13, 15]. Of the two isoprostanes measured, 8-iso $PGF_{2\alpha}$ was found to be elevated by about 30% in PE over NP. 5-$iPF_{2\alpha}$VI was not increased. In an untreated PE placenta, isoprostane expression would be expected to be increased 300% [11]. This attenuation of 8-iso $PGF_{2\alpha}$ and lack of increase in 5-$iPF_{2\alpha}$VI is again consistent with low-dose aspirin, which has been shown to decrease oxidative stress in pregnant women [15].

The omega-3 PUFAs EPA and DHA were found to be increased in EOPE. Omega-3 PUFAs are decreased in untreated PE women, which promotes platelet aggregation, vasoconstriction, and inflammation [20, 36]. These dietary lipids are susceptible to oxidation at their three double bonds, and their preservation indicates an attenuation of oxidative stress.

Of the non-COX products of AA, the HETE's were all elevated, with the largest percent increase of any eicosanoid occurring in 20-HETE (Figure 9). As HETE's are pro-inflammatory, and

20-HETE in particular is heavily implicated in hypertension, this demonstrates a basis for the pathogenesis of PE which is resistant to low-dose aspirin therapy (Specific Aim 1) [36, 37]. Similarly, 11,12-DHET and 14,15-DHET were found to be elevated and are suspected to have pro-inflammatory effects [41].

B. Sphingolipids

The balance of sphingolipids, particularly S1Ps, C1Ps, and ceramide, presents an interconnected manner for which cell fate is regulated through the mediation of inflammation, apoptosis, and immune function [43, 44, 46, 48]. It has been established that the placentas of PE women are characterized by increased apoptosis [9]. Sphingolipid metabolism should not be affected by low-dose aspirin therapy. For the 18 carbon chain length variants of S1P, sphingomyelin and ceramide, which can be interconverted, an increase was observed (Figure 8). As multiple signaling pathways converge on the sphingolipid "rheostat," the increases in free sphingolipids may be involved in the development of PE and would be unaffected by low-dose aspirin.

C. Gestational Age

The most apparent trend stratifying the LOPE and EOPE groups are that the LOPE group demonstrated values most similar to the NP group. Of the significantly different lipids, 13 of 25 showed a significant difference from NP to EOPE but not for NP to LOPE. For the C1P variant

C24:0, the opposite was true; LOPE showed a significant difference from NP but EOPE did not. For the remaining 11 of 25 significantly altered lipids, statistical significance was found for all categories compared to NP. This signifies that the EOPE category demonstrated more dramatic alterations in lipids while the LOPE category did not always reflect those same changes. As a further example, the pro-apoptotic ceramides were, on average, conversely increased in EOPE while decreased in LOPE. The conclusion of this pattern is that the women who delivered before 37 weeks of gestational age were those with the most severe PE, for which their delivery was becoming medically necessary for the health of the mother and the child. Correlating the severity of PE with the extent to which lipids were altered allows the inference of how those lipids may then be used to anticipate PE.

D. Fingerprint of Lipid Changes in Preeclampsia

As demonstrated in the radar graphs (Figure 6, Figure 7), there are patterns by which a fingerprint of the lipids significantly altered in PE can be visualized. Consideration for those lipids which were significantly similar was not done, as the similarity of a PE placenta to a NP placenta would provide no application diagnostically.

Superimposition of an individual woman's placental lipid productions may, conceivably, be projected in a blind manner onto the expected values for those lipids which have shown significant alterations in PE. This projection could then yield a general probability of that

woman's likelihood of having experienced PE during her pregnancy. A model for this prediction would rely on the fingerprint of the bioactive lipids. Clinical use of such a model would rely on both early and frequent sample collection in those patients at high risk for PE. Any prediction of progression to PE would be best served with venous blood sampling and/or urine collection, which are considered non-invasive.

E. Conclusions

Some women in VCU's high-risk clinic developed PE despite the established reduction in prevalence and morbidity of PE with low-dose aspirin therapy. The results of this study may explain why. HETEs, which mediate inflammation and hypertension, were significantly increased. As HETEs are produced by lipoxygenase enzymes rather than cyclooxygenase, they cannot be reduced by low-dose aspirin. Additionally, significant increases in sphingolipids convey a pro-inflammatory, pro-apoptotic state and are not affected by aspirin. Despite the apparent beneficial effects of aspirin on reducing pro-inflammatory eicosanoids, this study demonstrated there are pro-inflammatory lipids still being produced at significant levels in women who developed PE (Specific Aim 1).

The chief treatment for the management and prevention of PE, low-dose aspirin, was predicted to have demonstrable effects on thromboxane and prostacyclin. This was accurately reflected in the increase in prostacyclin metabolite 6-keto $PGF_{1\alpha}$, which is known to be preserved with low-dose aspirin therapy. Similarly, a lack of the anticipated 7-fold elevation of TXB_2 which occurs in untreated PE is consistent with the selective reduction in thromboxane

production that low-dose aspirin causes. Attenuation in oxidative stress can be inferred from the diminished increase in isoprostanes compared to untreated PE placentas and the increase in omega-3 PUFAs (Specific Aim 4).

The altered lipid profile of PE placentas provides both statistical significance for a large portion of the lipids analyzed (Specific Aim 3) and for anticipation of the severity of the disease state by the detrimental impact of that disease state on gestational age (Specific Aim 2).

As the UO1 grant continues, analysis of lipids will be done for longitudinal urine and plasma samples from the patients for which placentas were analyzed. In addition to investigating the use of lipid changes as a manner for fingerprinting the disease state in plasma and urine, further data will allow a correlation between placental lipid production and circulating lipids to be more readily made. Though mechanistic rationale of the placenta as a mediator for PE is abundant, further inference into causation will be permitted if the same lipids are found to be elevated in placental media, maternal plasma, and maternal urine.

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Appendix

Table 2. For analyzed lipids, calculated p-value is listed. For bolded p-values and their corresponding lipid, significance ($p < .05$) was achieved.

Lipid	**NP vs. PE**	**NP vs. LOPE**	**NP vs. EOPE**
EPA	.0739	.9534	**.0028**
DHA	**.0011**	.0565	**.0001**
Resolvin D_1	.1815	.4162	.5082
Resolvin D_2	.7523	.8584	.7749
AA	.6127	.6963	.7000
DHGLA	**.0019**	**.0310**	**.0012**
6-keto $PGF_{1\alpha}$	**.0008**	**.0086**	**.0017**
8-iso $PGF_{2\alpha}$	**.0361**	.1851	**.0405**
PGE_1	.2475	.2575	**.0162**
PGE_2	.0581	.5606	**.0181**
$PGF_{2\alpha}$	.1480	.4464	.1711
PGD_2	.5572	.9405	.4102
PGA_2	.5677	.7385	.7322
15d-PGJ_2	.7121	.8974	.4704
TXB_2	.1078	.7999	.0552

5-iPF$_{2\alpha}$-VI	.5377	.7262	.5551
5 HETE	**.0343**	.3018	**.0184**
12 HETE	.3288	.5253	**.0045**
15 HETE	**.0007**	.0539	**.0001**
20 HETE	**.0001**	**.0033**	**.0001**
LTD_4	.1706	.9943	.3857
LTC_4	.1048	.2513	.1651
LTE_4	.4161	.7895	.1248
LTB_4	.3798	.9984	.0952
Lipoxin A_4	.2668	.5929	.2736
8,9-EET	.6414	.7384	.6489
14,15-EET	.2369	.8162	.1191
8,9-DHET	.1459	.6468	.0741
11,12-DHET	**.0001**	**.0057**	**.0001**
14,15-DHET	**.0002**	**.0175**	**.0001**
C14:0 Ceramide	**.0127**	**.0229**	.3052
C16:0 Ceramide	.7209	.7872	**.0010**
C18:1 Ceramide	.3160	.0700	.0541
C18:0 Ceramide	.4486	.6531	**.0023**
C20:0 Ceramide	.7601	.6359	.0798
C22:0 Ceramide	.4447	.1526	.8322
C24:1 Ceramide	.9292	.1325	.2856
C24:0 Ceramide	.8101	.3164	.6002
C26:1 Ceramide	.1840	.1664	.5168
C26:0 Ceramide	.4025	.5589	.4797

C14:0 Monohexosylceramides	.2849	.1760	.9999
C16:0 Monohexosylceramides	.6134	.6388	.7612
C18:1 Monohexosylceramides	**.0004**	**.0064**	**.0002**
C18:0 Monohexosylceramides	.6373	.9075	.5621
C20:0 Monohexosylceramides	.7704	.9094	.7310
C22:0 Monohexosylceramides	.9002	.2287	.0540
C24:1 Monohexosylceramides	.3943	.8565	.0587
C24:0 Monohexosylceramides	**.0025**	.1626	**.0135**
C26:1 Monohexosylceramides	.1937	.2445	.9937
C26:0 Monohexosylceramides	.9544	.5646	.3590
C14:0 Sphingomyelin	.8068	.5512	.0549
C16:0 Sphingomyelin	.4912	.5902	.7053
C18:1 Sphingomyelin	.4413	.7305	.4089
C18:0 Sphingomyelin	**.0022**	.1061	**.0001**
C20:0 Sphingomyelin	.2420	.5867	.2036
C22:0 Sphingomyelin	.0885	.5127	**.0399**
C24:1 Sphingomyelin	.2985	.7011	.2333
C24:0 Sphingomyelin	.3424	.5307	.4224
C26:1 Sphingomyelin	.4540	.9844	.2567
C26:0 Sphingomyelin	.6739	.7382	.7854
C14:0 Ceramide 1-Phosphate	**.0172**	**.0463**	**.0043**
C16:0 Ceramide 1-Phosphate	.3112	.3312	.5853
C18:1 Ceramide 1-Phosphate	.3211	.4170	.5574
C18:0 Ceramide 1-Phosphate	.7286	.6690	.8999
C20:0 Ceramide 1-Phosphate	.0896	.8380	**.0064**

C22:0 Ceramide 1-Phosphate	.0709	.0901	.2966
C24:1 Ceramide 1-Phosphate	.0810	.1540	.2021
C24:0 Ceramide 1-Phosphate	**.0021**	**.0164**	.1372
C26:1 Ceramide 1-Phosphate	.5125	.2588	.7122
C26:0 Ceramide 1-Phosphate	.5850	.6855	.1211
C18:1 Sphingosine-1-Phosphate	**.0092**	.0683	**.0011**
C18:0 Sphingosine-1-Phosphate	.2192	.8617	**.0020**