Accumulation of Dietary and Non-Enzymatic Oxysterols in Preeclamptic Placentas: Insights into Cholesterol Dynamics in Pregnancy Complications

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Abstract

Pregnancy involves a series of intricately coordinated events, including decidualization, placentation, and parturition, with oxidative stress playing a central role in both normal and pathological pregnancy progression. Placental complications in pregnancy, including preeclampsia, characterized by hypertension and proteinuria after 20 weeks of gestation. Excessive ROS production disrupts cell physiology, affecting bioactive lipids such as cholesterol. Cholesterol's oxidative derivatives, oxysterols, function as active biological molecules regulating cholesterol synthesis and immune function. Oxysterols arise from both enzymatic and non-enzymatic pathways; non-enzymatic oxysterols, including dietary sources, may amplify oxidative stress under pathological conditions. This study aims to characterize dietary and non-enzymatic oxysterol presence in the human placenta, analyzing profiles in uncomplicated and preeclamptic pregnancies delivered preterm and at term. Findings indicate significantly higher oxysterol levels in preeclamptic placentas, particularly for 7-ketcholesterol (7-Keto) and 5α,6β-cholestanetriol, (Triol) regardless of gestational age. 5β,6β-epoxycholesterol (5β,6β-Epoxy) was elevated in term preeclampsia, while total cholesterol levels were notably lower in preeclamptic placentas. Oxysterols levels and cholesterol concentrations positively correlated with certain oxysterols yet inversely with others, highlighting unique oxidative signatures in preeclamptic conditions. These insights reveal oxysterols as potential biomarkers for preeclampsia and deepen our understanding of oxidative processes in pregnancy complications.

Keywords: dietary oxysterols, fatty acids, placenta disorders, cholesterol, lipid oxidation

Introduction

Preeclampsia is a pregnancy complication characterized by both new-onset hypertension plus new-onset proteinuria ≥ 300 mg/24 h after 20 weeks of gestation. Concomitant preeclampsia traits include endothelial dysfunction, hypertension, and end-organ damage. While the etiology of preeclampsia is not clear, evidence suggests that oxidative stress during pregnancy is implicated in both the normal development of the placenta as well as in the pathophysiology of complications such as miscarriage, premature rupture of the membranes (Jauniaux, Poston, & Burton, 2006) and pre-eclampsia (Burton & Yung, 2011).

Physiologically, lipid parameters, including cholesterol, rise during pregnancy over the course of pregnancy (Bartels & O'Donoghue, 2011), however, the generation of excessive oxidative species may have deleterious effects on cell physiology (Chiarello et al., 2020) Reactive oxygen and nitrogen species (ROS, RNS) can hinder the physiological function of cell membrane lipids by the oxidation of fatty acids, phospholipids, and cholesterol. Fatty acids play a critical role in determining structural and metabolic functions of the cell, particularly polyunsaturated fatty acids (PUFAs) of the omega-3 or omega-6 series such as linoleic acid (LA, omega 6) and α-linolenic (ALA, omega 3) considered essential fatty acids. LCPUFAs and their metabolites are required at various stages in placental development . However, an impaired materno-fetal LCPUFA transport is known to occur in pregnancies that are complicated and characterized by an abnormal placental function such as preeclampsia(Hanebutt, Demmelmair, Schiessl, Larqué, & Koletzko, 2008). Cholesterol is the major component of mammalian cell membranes and the precursor of vitamins, bile acids, and steroid hormones - some of which are synthetized by the human placenta - and its abundance in tissue is determined by endogenous synthesis and dietary intake. The placenta regulates cholesterol biosynthesis, metabolism, and transport and exchange between the maternal and fetal compartments (Palinski, 2009).

Oxysterols are cholesterol oxidation products formed due to the cholesterol's susceptibility to oxidation and differ from the parent cholesterol by the presence of an additional ketone, epoxy, or hydroxyl group in the A or B rings or a hydroxyl group in the side chain (Medina-Meza, Rodriguez-Estrada, Lercker, Soto-Rodriguez, & Garcia, 2011). Oxysterols are biologically active molecules, they regulate cholesterol biosynthesis (Bjorkhem, 2002) and act as ligands to G protein-coupled receptors (Vurusaner, Leonarduzzi, Gamba, Poli, & Basaga, 2016) and nuclear receptors (Aye, Waddell, Mark, & Keelan,

2011). They can also affect the immune system by suppressing the production of IgA by B cells (Spann & Glass, 2013). While Oxysterols have been reported in amniotic fluid, maternal blood, and placental tissue with mixed results (Bodzek, Janoszka, Wielkoszynski, Bodzek, & Sieron, 2002), a single study has investigated the presence of the oxysterol 27-hydroxycholesterol in the human placenta (Winkler et al., 2017).

Oxysterols can be generated both enzymatically or non-enzymatically, where enzymatic pathways are physiologically regulated, and non-enzymatic pathways may occur randomly and are not regulated. While the formation of enzymatic oxysterols is mediated by cytochrome P450 family (Iuliano, 2011; Zmyslowski & Szterk, 2019) non-enzymatic oxysterols can be formed from cholesterol autooxidation in foods due to storage, heat, and processing. 7-ketocholesterol (7-Keto), 7β-hydroxycholesterol (7β-OH), 5α,6α-epoxide (5,6α-e poxy), 5β,6β-epoxide (5,6β-epoxy), cholestan- 3β,5α,6β-triol (triol), but also part of 7α-OH and 25-OH-C are the most abundant oxysterols reported in foodstuff (Lisaura Maldonado-Pereira, Carlo Barnaba, & Ilce Gabriela Medina-Meza, 2023). Both altered cholesterol levels and induction of their oxidized metabolites have been confirmed in several pathologies (Sottero et al., 2019; Zmyslowski & Szterk, 2019).

An increase in lipid peroxidation products has been reported in maternal serum of healthy pregnancies (Maseki, Nishigaki, Hagihara, Tomoda, & Yagi, 1981) . While this phenomenon seems to be balanced by increasing the activity of antioxidant systems during pregnancy (Wang, Walsh, Guo, & Zhang, 1991), this antioxidant capacity seems to be reduced in women with preeclampsia (Basu et al., 2002). It is plausible that this results in an imbalance between oxidation and antioxidation reactions towards oxidative stress where the placenta becomes the main target. Although oxysterols have been implicated in the pathogenesis of several disease states including atherosclerosis (Zmyslowski & Szterk, 2017) and dementia (Testa et al., 2016), few studies have characterized oxysterol concentrations during pregnancy or examined their relationship with pregnancy outcomes (Rideout et al., 2023). In this study, we aimed to quantify cholesterol, fatty acids, and the accumulation of both dietary oxysterols (DOxS) and non-enzymatic oxysterols in the human placenta and evaluate their association with preeclampsia. .

Materials and Methods

Ethics, exclusion criteria, and placenta collection

The institutional review boards at Michigan State University and Sparrow Hospital approved the project. Women admitted for C-section delivery at Sparrow Hospital (Labor & Delivery Service) were asked to participate in the study and provided with written informed consent (IRB IRB#17-1359M). Human placentas (n = 27) were obtained from three groups of patients previously described (Sethuraman et al., 2022), 1) healthy term pregnancies (Term; n = 12), 2) term preeclamptic pregnancies (Term PE; n = 8) and 3) pre-term preeclamptic pregnancies (Pre-Term PE; n = 8). Maternal and neonatal anthropometric and clinical data from the mother's and infants' medical records were obtained. Healthy-term pregnant mothers (at ≥ 37 weeks of pregnancy) with no complications and with none of the exclusion criteria (see below) were enrolled as healthy-term controls. Mothers with a diagnosis of preeclampsia (at ≥ 36 weeks of pregnancy) were enrolled in the term preeclampsia group, while mothers with a diagnosis of preeclampsia (between 28 to 35 weeks of pregnancy) were enrolled in the preterm preeclampsia group. Preeclampsia was defined by the following American Congress of Obstetricians and Gynecologists criteria (ACOG, 2019): 1) systolic blood pressure of 140 mmHg or more or diastolic blood pressure of 90 mmHg or more on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure and 2) proteinuria. Proteinuria was defined as a 24-h urine protein of >300 mg or protein to creatinine ratio of >0.3. Subjects with the following conditions were excluded from the study: multiple pregnancies, clinical chorioamnionitis, chromosomal anomalies, diabetes (gestational diabetes, type 1 diabetes, and type 2 diabetes), chronic hypertension (hypertension before pregnancy), gestational hypertension without the diagnosis of preeclampsia, major cardiovascular diseases, such as myocardial infarction, cholestasis, drug abuse, and smoking. All placentas were processed within 1 h of delivery. A core biopsy ~2 cm away from the periphery and the umbilical cord attachment was collected and kept frozen until further processing for mRNA and protein expression analyses. Placental tissues were stored at -80 °C for further analysis.

Materials, Chemicals and Reagents

Methanol was purchased from Sigma-Aldrich (St. Louis, MO), chloroform from Omni Solv (Burlington, MA), hexane from VWR BDH Chemicals (Batavia, IL), 1-butanol and potassium chloride (KCl) from J. T. Baker (Allentown, PA), diethyl ether from Fisher Chemical (Pittsburgh, PA), and sodium sulfate anhydrous (Na_2SO_4) and sodium chloride (NaCl) from VWR. Labeled standards of cholesterol *d-7*, 7α-hydroxycholesterol-*d-7* (7α-OH), 7β-hydroxycholesterol-*d7* (7β-OH), 5,6α-epoxycholesterol-*d7* (5,6α-Epoxy), 5,6β-epoxycholesterol-*d7* (5,6β-Epoxy), 25-hydroxycholestero-*d6* (25-OH) cholestane-

3b,5a,6b-triol-*d7* (triol), 7-ketocholesterol-*d7* (7-Keto), 24(S)-hydroxycholesterol-d7 *(24-OH* were purchased from Avanti Polar® (Birmingham, AL), 4β- hydroxycholesterol-*d7)*, 25(S)-27-hydroxycholesterol (27- OH) from Cayman chemicals® (Ann Arbor MI) and 6 ketocholestanol (6-Keto), 3,6 Dione, 20α-hydroxycholesterol (20-OH), from Steraloids (Newport, R.I.) and purified by using aminopropyl (NH_2) cartridges (500 mg/3 mL) from Phenomenex® (Torrance, CA).

Lipid extraction

Before lipid extraction, placenta tissues were thawed, within 4h, and washed three times with phosphate-buffered saline (PBS) to remove any contaminating blood. Then, approximately 200 mg of placenta were placed in a mortar, liquid nitrogen was added, and the sample was gently minced until a fine powder, then transferred to a glass tube with a screwcap. 50 µg of 19-hydroxycholesterol and 140 µg of 5α-cholestane plus a mixture of the OxyS deuterated standards (1 ng/µL each) in ethanol with 0.1% of BHT were added as internal standards for the determination of total cholesterol and OxyS respectively. Then, lipid fraction was extracted with 3 mL of hexane/isopropanol (4/1, v/v) with 0.1% of BHT for 3 min, following a sonication for 10 min in cold water. The homogenate was then centrifuged at 2,800 rpm for 10 min at 4 °C. The supernatant was transferred to a new glass tube and the extraction was repeated with the remaining pellet. The supernatants from the two extractions were pooled.

Total Cholesterol

A quantification method for cholesterol and oxysterols previously developed in our laboratory (Kilvington, Barnaba, Rajasekaran, Laurens, & Gabriela Medina-Meza, 2021; L. Maldonado-Pereira, C. Barnaba, & I. G. Medina-Meza, 2023) was used in this study. Subsequently, cold saponification was done with 1 N KOH solution in methanol left under gentle agitation at a cool temperature, and covered from the light overnight. One-tenth of the unsaponifiable matter was subjected to silylation. The sample was mixed with 100 µL of pyridine and 100 µL of Sweeley's reactive mixture (pyridine/hexamethyldisilazane/trimethylchlorosilane, 10:2:1, v/v/v) at 75°C for 45 min, dried under nitrogen stream and dissolved in 1 mL of n-hexane. One microliter of the silylated solution was injected into a gas chromatograph using a fitted with a Zebron™ZB-5HT (Phenomenex, Torrance, CA) capillary column (30 m × 0.25 mm × 0.25 µm). Oven temperature conditions were set up as follows: 260 to 300ºC at 2.5◦C/min, then from 300 to 320◦C at 8ºC/min, and finally hold at 320ºC for 1 min. The injector and

detector were both set at 320°C. Helium was used as the carrier gas at a flow rate of 54.0 mL/min, a split ratio at 1:50, and a pressure constant of 134 kPa.

Oxysterols Quantification

The other nine-tenths of the sample was used for the quantification of oxysterols. Purification by NH_2-SPE was performed as previously reported (Lisaura Maldonado-Pereira et al., 2023). The enriched fraction was recovered and evaporated to dryness under a nitrogen stream. Subsequently, the purified fraction was silylated with Sweeley's reactive mixture (at 75 °C for 45 min), dried under a nitrogen stream, and dissolved in 200 µL of n-hexane. OxyS were isolated and identified using the selected ion molecule (SIM) method with a GCMS-QP2010 SE single quadrupole Shimadzu Corporation (Kyoto, Japan). One µL of the silylated OxyS was injected into a single quad GCMS-QP2010 SE with the following conditions: from 250 to 280 °C at 2°C/min, hold at 280 °C for 7 min, and from 280 to 315 °C at 1.5 °C/min. Helium was used as a carrier gas (flow rate of 0.37 mL/min); the split ratio was 1:15 and the pressure was 49.2 KPa. The interface temperature for the GC-MS was 320 °C, with the electron multiplier voltage set at 70 kV and the injector at 320 °C. A fused silica column (30 m x 0.25 mm i.d. x 0.25 um thickness) coated with 5% phenyl polysiloxane (Phenomenex Zebron ZB-5) was used.

Fatty acids methyl esters (FAME) profiling

Fatty acid methyl esters (FAME) were prepared according to the transesterification described somewhere else (Maldonado-Pereira, Barnaba, de Los Campos, & Medina-Meza, 2022). One µL of the methylated sample was injected into a gas chromatograph (GC 2010 Shimadzu®, Kyoto Japan) equipped with an autosampler (AOC- 20s Shimadzu®) and a flame ionization detector (FID).

A fused silica DB-WAX (Agilent J&W, Santa Clara, CA) capillary column (30 m × 0.32 mm i.d. × 0.25 µm film thickness) was used to separate the fatty acids. The oven was programmed as follows: 120 °C to 200°C with a rate of 3°C/min, then from 200 °C to 240°C with a rate of 2 °C/min and held for 2 min. The injector's pressure was set up at 92.2 kPa. Temperature for both, injector and detector were set at 250°C. H_2 was used as carrier gas at 1 mL/min and the split ratio was 5.0. Data acquisition was done on LabSolutions software version 5.106 (Shimadzu®, Kyoto, Japan) and peak areas were identified by comparing their retention times to the pure standards (FAME 37 mixture). Fatty acid content were quantified using tridecanoic acid as the internal standard and reported as g of fatty acid per 100 g of sample. Results on fatty acids contents were obtained using the sums: Σ saturated fatty acids (Σ SFA = C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 +

C22:0 + C23:0 + C24:0); Σ monounsaturated fatty acids (Σ MUFA = C14:1, cis-9 + C15:1, cis-10 + C16:1, cis-9 + C17:1, cis-10 + C18:1, cis- 9 + C20:1, cis-11 + C22:1, cis-13 + C24:1, cis-15); Σ polyunsaturated fatty acids (Σ PUFA = C18:2, cis-9,12 + C18:3, cis-6,9,12 + C18:3, cis-9,12,15 + C20:2, cis-11,14 + C20:3, cis-8,11,14 + C20:3, cis-11,14,17 + C20:4, cis-5,8,11,14 + C22:2, cis-13,16 + C20:5, cis-5,8,11,14,17 + C22:6, cis-4,7,10,13,16,19). and Σ trans fatty acids (Σ TFA= C18:1, trans-9 + C18:2, trans-9,12).

Statistical Analysis

Descriptive statistics were performed, and both mean, and confidence intervals (95%) were computed. Since data was not normally distributed, the variable was log-transformed for all analyses. The relationship of individuals' oxysterols with serum cholesterol and maternal BMI was assessed with Pearson correlation coefficients. Associations between maternal Oxys and fatty acids and terms were assessed using multivariable linear regression. We considered nulliparity (yes/no), birth weight, gestation age, maternal age, and pre-pregnancy BMI (continuous variables) as potential confounding factors. Confounders with a (p-value < 0.05) were considered significant and were further adjusted in the models. All analyses were conducted using SPSS version 29 (IBM(R)). Statistical significance was set at a two-sided alpha level of 0.05. When comparing data that did not follow a normal distribution after a density plot analysis and was not homogenous after a residual plot analysis a Statistical differences between food categories were evaluated by using the non-parametric Kruskal-Wallis ANOVA by Ranks test, at $p < 0.05$ significance level.

Results and discussion

Clinical Characteristics

Maternal and neonatal clinical data are shown in **Table 1**. No significant differences were observed in maternal age, height, weight, SROM score, or neonatal birth weight percentiles among groups. Maternal BMI was lower in the Term PE group when compared with the Pre-Term PE group. Neonates' birth weight was lower in the Pre-Term PE group compared to the term and Term PE groups.

Table 1. Clinical characteristics

	Term (n = 12)	Term PE (n = 7)	Pre-Term PE (n = 9)

	≥ 37 weeks		≥ 36 6/7 weeks		28-35 weeks	
	Mean ± SEM, percent, or N	**Range**	**Mean ± SEM, percent or N**	**Range**	**Mean ± SEM, percent, or N**	**Range**
Age (years)	31.8 ± 1.0[a]	27 - 39	25.6 ± 3.0[b]	19 - 41	27.9 ± 1.1[a,b]	24 - 34
Height (m)	1.7 ± 0.02	1.6 - 1.8	1.6 ± 0.03	1.5 - 1.7	1.6 ± 0.02	1.5 - 1.7
Maternal weight (kg)	87.8 ± 5.6	68.5 - 120.2	76.4 ± 11.9	47.6 - 137.0	95.5 ± 8.7	68.0 148.8
BMI (kg/m^2)	29.6 ± 2.1	16.6 - 46.8	29.4 ± 4.0	20.5 - 50.2	36.9 ± 3.2	28.3 - 56.3
Birth weight (g)	3,648 ± 105[a]	3,090 - 4,240	3,081 ± 220[a]	1,980 - 3,540	2,301 ± 228[b]	1,460 - 3,460
Birth weight percentile	63.5 ± 6.1	30.8 - 99.6	43.7 ± 14.0	1.8 - 89.7	60.2 ± 8.4	20.3 - 95.2
Newborn sex (Female/Male)	(4/8)		(3/4)		(3/6)	
SROM (%)	0 %		42 %		22 %	

Data expressed in mean ± SEM or percent unless otherwise specified. IQR: interquartile range, PE: preeclampsia, Pre-Term PE: preterm preeclampsia, Term PE: term preeclampsia, PTL: preterm labor, SROM: spontaneous rupture of membranes, and T: term control. a ≠ b denotes significant differences between groups.

Placental fat and fatty acids profile

The fatty acids profiles on human placentas for all three groups are reported in **Table S1.** A total of 31 fatty acids were detected in all plcanta groups, whereas heneicosylic acid (C21:0) was only detected in healthy term placentas. No significant differences within the profile of fatty acids were noted across the three groups. However the ratio of elaidic acid (C18:1 cis9) *vs*. C18:1 trans9 was significantly increased in preeclamptic placentas. IAberrant fatty acid metabolism is known to be involved in the pathogenesis of preeclampsia (Wadhwani, Patil, & Joshi, 2018). Trans fatty acids come from processed foods and are transferred from the mother to the fetus. In addition, both the fetus and the breastfed baby report maternal intake of trans fatty acids (Basak, Vilasagaram, & Duttaroy, 2020; Cohen, Rifas-Shiman, Rimm, Oken, & Gillman, 2011).

Surprisly, pentadecanoic acid (C17:0) an essential odd-chain saturated fatty acid was significantly lower different ($p<0.05$) in preeclamptic placentas independent of the term. C17:0 is being suggested as marker of dairy fat intake (Jenkins, West, & Koulman, 2015), this odds fatty acids are taken up by the animal from the rumen and utilized by the mamary glad fot milk fat production. Also, pentadecylic acid (C15:0) along with C17:0 have been associated with reduced risk for developing multiple sclerosis due

to the increase of membrane fluidity (Holman et al., 1995). Our results are in cronstrast with literature regarding the abundance of C17:0 over C15:0 on biofluids and adipose tissue (C15:0 of 0.32%, C17:0 of 0.34%) (Brevik, Veierod, Drevon, & Andersen, 2005), erythrocyte (C15:0 of 0.28%, C17:0 of 0.45%) and human hindmilk (C15:0 of 0.46%, C17:0 of 0.57%) (Valentine, Hurst, & Schanler, 1994).

Total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), and total PUFAs are significantly reduced ($p < 0.05$) in placentas with PE independent of gestational age. Our results are in agreement with previous work which reports lower maternal LCPUFA levels in early pregnancies (26-30th week) with preeclampsia (Wadhwani et al., 2014). These differences could be due to poor dietary sources, inhibitions of synthesis, or decreased release from maternal stores.

Maternal LCPUFAs are not only important for fetal neurodevelopment but also for optimal fetoplacental growth and development. The fatty acid requirement of the fetus depends on the maternal diet as well as on placental transport and metabolism (Haggarty, 2010). The human placenta is permeable to fatty acids and this transfer to the fetus is required for proper growth and development (Wadhwani et al., 2018). Since the conversion of LA or ALA to their LCPUFAs is not sufficient, the mother has to obtain these LCPUFAs from the diet as the fetus critically depends on the maternal supply of LCPUFAs. For example, it is estimated that 67−75 mg/day of DHA is accumulated *in utero* during the last trimester of gestation. Maternal supplementation with DHA up to 1 g/d or 2.7 g n-3 LCPUFAs did not have any harmful effect (Koletzko, Cetin, Brenna, & Grp, 2007). Furthermore, the trophoblast cells require LCPUFA from early pregnancy to carry out various processes associated with optimal placental growth and development (Rani, Chavan-Gautam, Mehendale, Wagh, & Joshi, 2015).

Cholesterol homeostasis dysregulated with placenta disorders

Cholesterol is fundamental for fetal development. In the placenta, cholesterol crosses two barriers (apical and balotareral membrane) to reach from the maternal to the fetal circulation. Cholesterol travels across the syncytiotrophoblast from the maternal lacunae, which expresses LDL receptors. ABCA and ABGA cholesterol transporters control the efflux of cholesterol into the fetal microcirculation as HDL particles (Palinski, 2009; Woollett, 2005). A significant reduction of total cholesterol in Term-PE placenta in comparison with Term ($p < 0.05$) was observed. This observation is in contrast with the literature that reports increased levels of cholesterol, triacylglycerides, HDL, and LDL in the second and

third trimesters of the pregnancies. However, the majority of reports measure these parameters in blood. These differences may be due that, in current obstetrics settings, free cholesterol is not measured in the placenta after delivery and there is not a reference range for “healthy” lipid parameters during normal pregnancy (Bartels & O'Donoghue, 2011). More studies in this regard should help to unveil lipids and cholesterol homeostasis during pregnancy.

Presence of oxysterols in the human placenta

In this study, we report for the first time the presence of 11 oxysterols in human placentas with preeclampsia at different terms **(Table 2).** Six OxyS (7-OH isomers, 5,6-isomers, 7 -keto, and Triol) were detected in all human placentas (n=28), representing up to 70% of the total oxysterol content in human placentas. The sum of all oxysterols was two-fold higher in preeclamptic placentas, independent of gestational age (PT-PE -251.08 ng/g- or Term-PE-273.83 ng/g**) (Figure 1).**

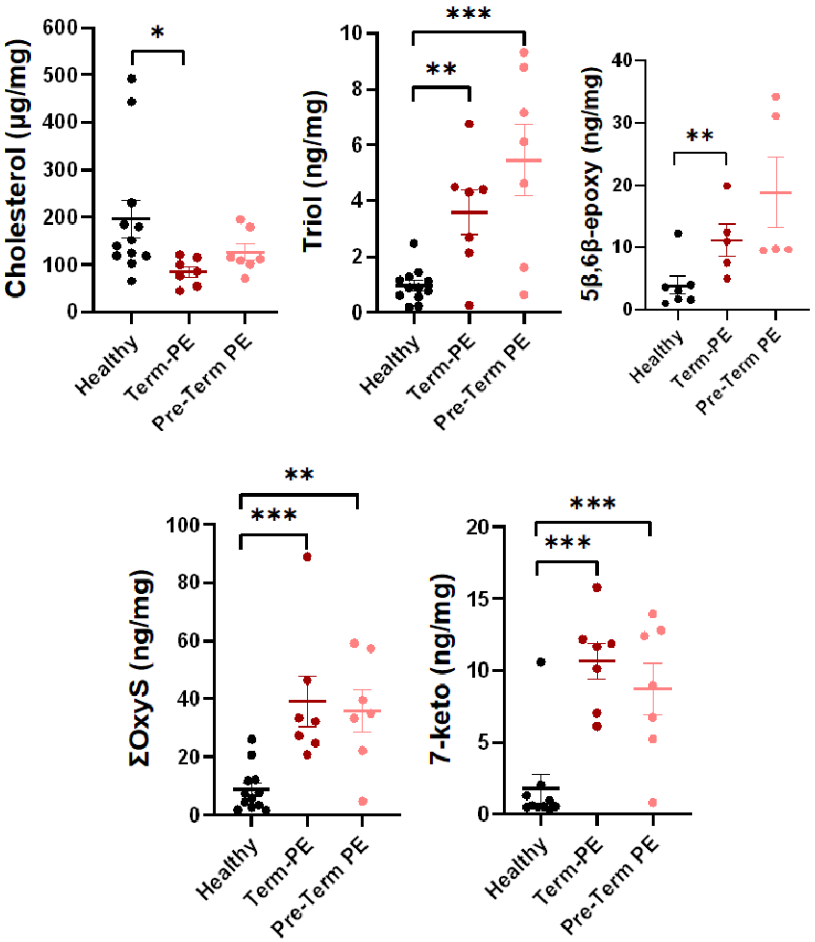

Figure 1. Mean (± SEM) of total OxyS (ΣOxyS), cholesterol, 7-Keto, Triol), 5α,6α-epoxy; 5β,6β-epoxy, 7α-OH and 7β-OH in human placentas derived from age-matched healthy or preeclamptic subjects at term (PE-Term) or preterm (PE-PTB; <37 weeks GA) *P<0.05, **P<0.005, ***P<0.0005 by ANOVA.

Similarly to reported oxysterol serum levels , cholesterol was significantly decreased in Term - PE whereas 7-keto, 5β,6 β-epoxy, and Triol were increased and positively correlated with the condition of preeclampsia independent of gestational age (term or preterm). These oxysterols are not enzymatic and can be generated by ROS or derived from diet . 5β,6β-epoxy was significantly higher in the term, but not preterm, preeclamptic placentas. 5,6-epoxy isomers content in the blood are significantly higher in pregnancy-induced hypertension women (p=0.017) along with the increase in total oxysterols (p=0.042) . These results agree with Sevanain et alwho found that especially 5β,6β epoxy no 5α,6α epoxy to be the most abundant in human plasma. On the contrary, reported two side chains oxysterols (27-OH 144.5 ng/mL > 24-OH 84.9 ng/mL) as the major oxysterols in blood during mid-term pregnancy followed by 7α-OH and 24-OH with 47.4 ng/mL and 18.4 ng/mL respectively. The authors, however, did not report epoxides in their study. More recently Dickson et al. (Dickson, Yutuc, Thornton, Wang, & Griffiths, 2022) found C-7 derived oxysterols as the major abundant in plasma as follows: 7β-OH > 7α-OH >26-OH and 24-OH. These discrepancies highlight the need for harmonized quantification methods and critical nutritional assessment to distinguish the origin of these oxysterols.

Table 2. Percent of oxysterol abundance in human placentas.

Oxysterol	ID	% Abundance in human placenta (n= 28)
7α-hydroxycholesterol	7α-OH	92.9
7β-hydroxycholesterol	7β-OH	92.9
cholesterol 3β,5α,6β-triol	Triol	92.9
7-ketocholesterol	7-Keto	85.7
cholesterol 5α,6α-epoxide	5α,6α-epoxy	64.3
cholesterol 5β,6β-epoxide	5β,6β-epoxy	60.7
25-hydroxycholesterol	25-OH	25.0
22-hydroxycholesterol	22R-OH	21.4
6-ketocholestanol	6-Keto	14.3
20S-hydroxycholesterol	20-OH	10.7
24-hydroxycholesterol	24-OH	7.1

Furthermore, we confirm the presence of 20S-hydroxycholesterol (20-OH) previously reported . This is an interesting oxysterol that has barely been reported in clinical studies despite being biologically active in vitro . 20-OH inhibits the SREBP-2 to its active form as the principal transcription factor regulating cholesterol biosynthesis. It has been hypothesized that 20-OH may bind to INSIG similarly to other side-chain oxysterols In addition, 20-OH, along with side-chain OxyS (22r-OH and 20R, 22R-dihydroxycholesterol) are ligands of LXRs .

Surprisingly none of the most abundant Oxys reported in dietary sources (5α,6α-epoxy, 7α-OH, and 7β-OH) significantly differ from the healthy term placentas. When analyses were conducted including maternal body weight as a covariate, significant differences in oxysterols among groups remained.

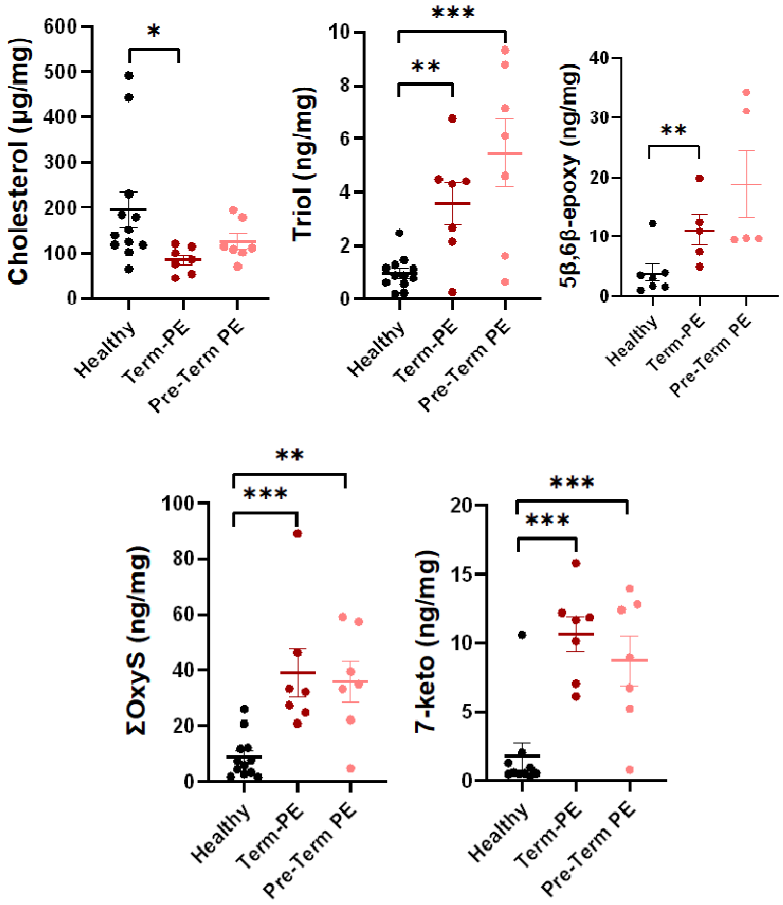

Figure 1. Mean (± SEM) of total oxysterols (ΣOxyS), cholesterol, 7-Keto, Triol, 5α,6α-epoxy; 5β,6β-epoxy, 7α-OH and 7β-OH in human placentas derived from age-matched healthy or preeclamptic subjects at term (PE-Term) or preterm (PE-PTB; <37 weeks GA) *P<0.05, **P<0.005, ***P<0.0005 by ANOVA.

Table 3. The ratio between individual oxysterols and the total oxysterol content in placental tissue.

Ratio	Control (n=12)		Pre-Term PE (n=9)		Term- PE (n=7)		*p-value*
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	
Total Oxys (ng/mg)	8.76	7.74	35.87	19.09	39.12	23.42	**0.001**
7α-OH/Oxys	0.21	0.06	0.10	0.13	0.04	0.02	**0.001**
7β-OH/Oxys	0.26	0.10	0.09	0.10	0.04	0.02	**0.001**
5,6β/Oxys	0.28	0.15	0.39	0.16	0.33	0.12	
5,6α/Oxys	0.03	0.01	0.07	0.04	0.05	0.03	
Triol /Oxys	0.19	0.14	0.16	0.08	0.13	0.10	
7-keto/Oxys	0.15	0.13	0.27	0.17	0.33	0.16	
25-OH/Oxys	0.04		0.17		0.13	0.10	
7α OH/7β OH	0.86	0.25	1.15	0.79	1.15	0.26	
5,6α Epoxy/5β Epoxy	9.84	6.24	5.97	2.07	7.96	6.03	

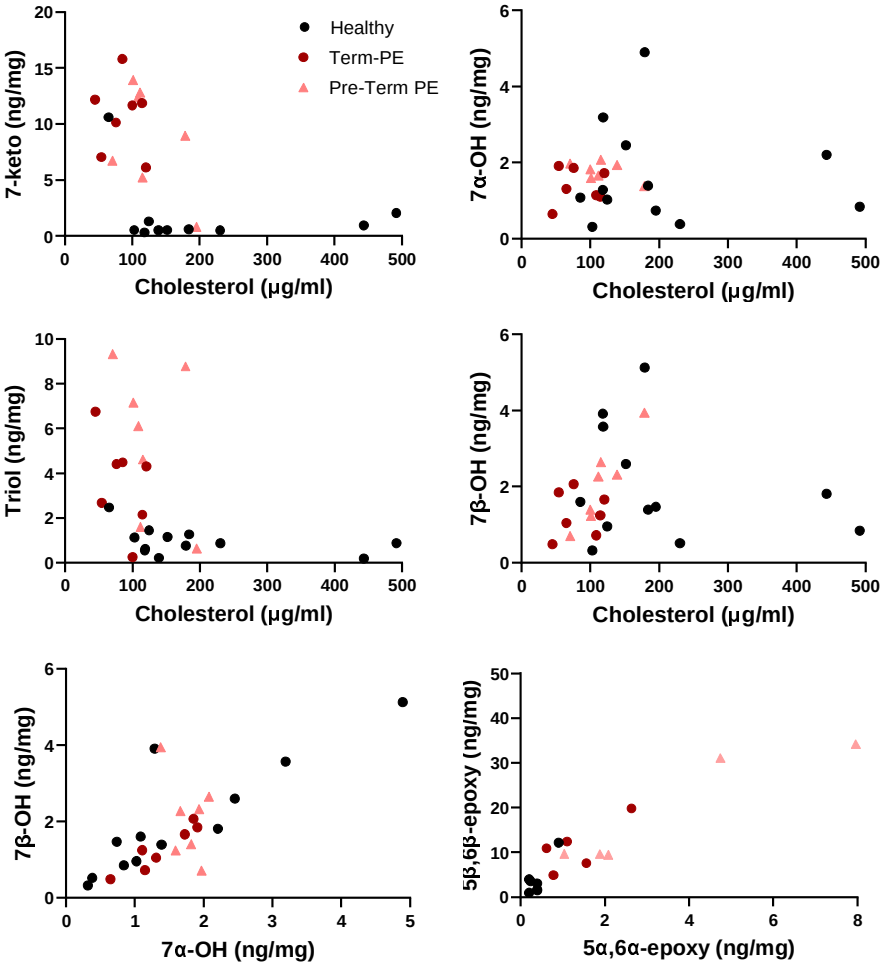

Figure 2. Correlation plots between oxysterols (OxyS) and cholesterol in human placentas derived from age-matched healthy or preeclamptic subjects at term (PE-Term) or preterm (PE-PTB; <37 weeks GA).7-Keto, Triol, 5β,6β-Epoxy7α-OH and 7β-OH.

Fatty acid associations with oxysterols

Several correlations among OxySs were detected when all three groups (Healthy, PE-Term, and PE-Term) are shown in **Figure 4.** This included a positive correlation between 7α-OH and 7β-OH (r^2: 0.741; p<0.01 and 0.548; P < 0.0001) and between 5α,6α-Epoxy and 5β,6β-Epoxy (r^2: 0.925;p< 0.01 and 0.855; P < 0.0001). Notably, cholesterol concentrations were positively correlated with 7α-OH and 7β-OH and negatively correlated with Triol and 7-Keto.

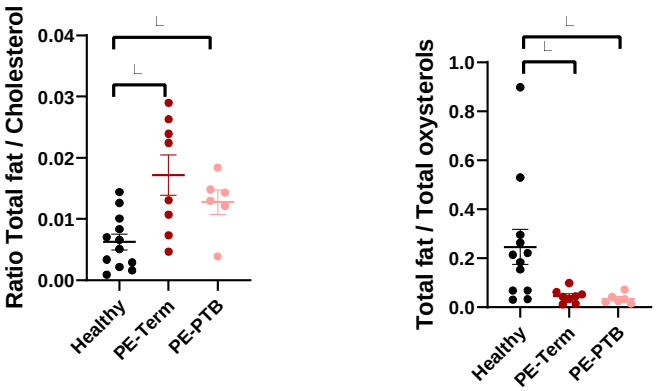

Figure 3. Correlation plots between total placental fat and OxyS and cholesterol (p<0.05) in human placentas derived from age-matched healthy or preeclamptic subjects at term (PE-Term) or preterm (PE-PTB; <37 weeks GA).

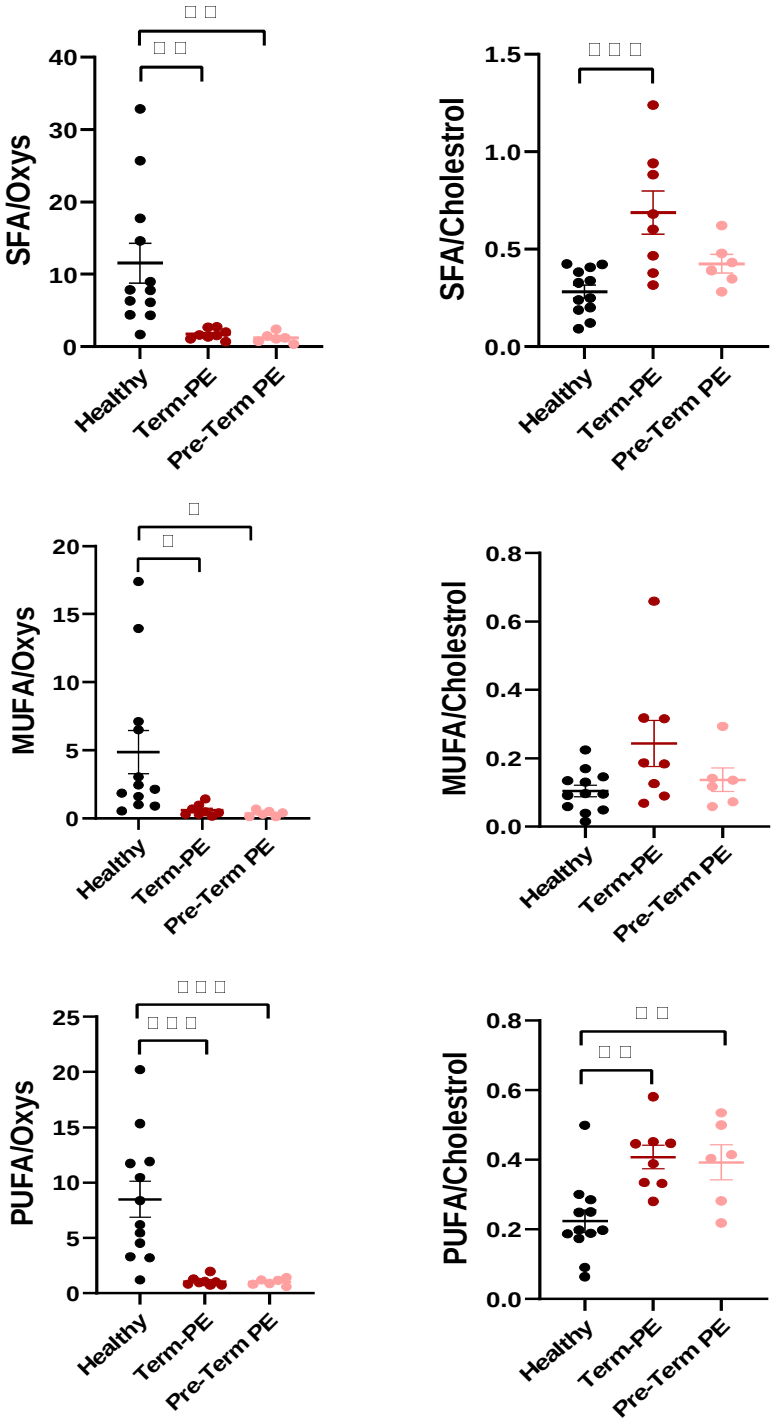

Figure 4. Correlation plots between saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFAs) with cholesterol and OxyS $p<0.05$ in human placentas derived from age-matched healthy or preeclamptic subjects at term (PE-Term) or preterm (PE-PTB; <37 weeks GA).

Conclusion

We present findings on the concentration of both DOxS and enzymatic Oxysterols in the human placenta. Given the limited sample size and reliance on self-reported data in this study, further investigations are needed to establish the use of oxysterols as biomarkers for pregnancy-related disorders. Unlike cholesterol, which is commonly monitored in non-pregnant adults, levels of cholesterol and oxysterols are not routinely assessed during pregnancy, and there are no universally accepted normal ranges throughout gestation. Nevertheless, emerging evidence suggests that elevated maternal cholesterol and oxysterols levels during pregnancy are associated with heightened risks of Pre-Term PE; however, further studies regarding the normal/safe ranges of cholesterol during pregnancy, the role of hypercholesterolemia and pregnancy-related cardiovascular disorders, incidence of preeclampsia and preterm delivery with oxysterols, the role of diet supplementation o reduce cholesterol in pregnancy are needed. Although our findings cannot provide causative evidence to support a direct role in maternal preeclampsia, the results confirm that during pregnancy increase in lipids occurs after the second trimester and oxysterols is included, increasing the overall sterol content. Profiling oxysterols in the placenta may be of potential utility as predictive biomarkers of preeclampsia or other pregnancy disorders.

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Author contributions

I. G. Medina-Meza: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; data curation; supervision and formal analysis. **A. Veiga-Lopes** writing, data curation, investigation; writing, review and editing; **L. Zou**, formal analysis and data curation, **L. Maldonado-Pereira**, formal analysis and data curation. **Elvis Ticiani,** statistics **Visalakshi Sethuraman**, patien recruitment, sample collection , **Isoken Nicholas Olomu**, patient recruitment, sample collection **Robert Long**, patient recruitment, clinical data abstraction.

Conflict of interest statement

The author declares no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

All data are included in the manuscript

SUPPLEMENTAL INFORMATION

Table S1. Oxysterols and fatty acids profile in human placenta

Metabolite	Control (n=12)		Pre-Term PE (n=9)		Term- PE (n=7)		
	mean	SD	mean	SD	mean	SD	p-value
Cholesterol ug/mg	195.81	134.31	126.04	44.49	85.14	28.88	**0.05**
7alpha (ng/mg)	1.65	1.34	1.78	0.25	1.39	0.47	
7beta (ng/mg)	2.01	1.49	2.08	1.08	1.30	0.59	
5,6beta (ng/mg)	3.86	3.85	18.84	12.67	11.15	5.68	**0.01**
5,6alpha (ng/mg)	0.39	0.25	3.54	2.83	2.80	3.66	
Triol (ng/mg)	0.97	0.61	5.46	3.36	3.58	2.08	**0.001**
7keto (ng/mg)	1.80	3.14	8.70	4.77	10.69	3.30	**0.001**
25-OH ug/mg)	0.28		3.73		7.75	10.76	
22-OH (ng/mg)	0.56	0.36	2.17		2.17	1.48	
20-OH (ng/mg)	0.41		2.12		1.74		
6-keto (ng/mg)			0.79	0.70	0.87	0.28	
24-OH (ng/mg)			3.42		33.80		
Total Oxys (ng/mg)	8.76	7.74	35.87	19.09	39.12	23.42	**0.001**
7a/Oxys	0.21	0.06	0.10	0.13	0.04	0.02	**0.001**
7bOH/Oxys	0.26	0.10	0.09	0.10	0.04	0.02	**0.001**
5,6beta/Oxys	0.28	0.15	0.39	0.16	0.33	0.12	
5,6alpha/Oxys	0.03	0.01	0.07	0.04	0.05	0.03	
Triol /Oxys	0.19	0.14	0.16	0.08	0.13	0.10	
7keto/Oxys	0.15	0.13	0.27	0.17	0.33	0.16	
25OH/Oxys	0.04		0.17		0.13	0.10	
7a OH/7b OH	0.86	0.25	1.15	0.79	1.15	0.26	
5a Epoxy/5b Epoxy	9.84	6.24	5.97	2.07	7.96	6.03	
C10	0.60	0.36	0.79	0.67	0.56	0.21	
C11	0.08	0.04	0.08	0.05	0.10	0.05	
C12	1.96	4.21	2.32	4.88	1.44	2.77	
C14	2.73	1.07	1.95	1.47	1.95	0.93	
C14:1n5	0.39	0.23	0.13	0.02	0.25	0.09	
C15	0.67	0.12	0.51	0.05	0.75	0.31	
C16	29.68	3.35	28.26	2.25	31.87	5.38	
C16:1n7	1.68	0.72	0.95	0.53	1.42	0.64	
C17	0.49	0.16	0.35	0.08	0.34	0.11	**0.042**
C17:1n7			0.01		0.06		
C18	11.56	2.20	10.39	3.21	10.73	3.10	
C18:1trans9	0.25	0.21	0.05	0.07	0.03	0.04	**0.009**
C18:1cis9	15.34	5.31	11.17	4.14	14.09	6.10	
C18:2tran6	0.04		0.01				
C18:2cis6	9.62	1.97	11.70	1.76	11.96	3.15	
C18:3n6	0.13	0.18	0.08	0.03	0.08	0.03	

C18:3n3	5.55	4.07	6.55	3.60	5.60	6.60	
C20	0.07	0.03	0.08	0.03	0.09	0.04	
C20:1n9	0.09	0.02	0.09	0.03	0.09	0.03	
C20:2n6	0.11	0.04	0.15	0.05	0.11	0.03	
C20:3n6	2.44	0.87	3.41	1.33	2.45	1.19	
C20:4n6	13.93	3.95	17.98	4.30	14.05	5.95	
C21	1.33						
C20:5n3	0.06	0.03	0.08	0.05	0.06	0.02	
C22	0.08	0.03	0.09	0.08	0.08	0.04	
C22:1n9	0.04	0.04	0.04	0.02	0.03	0.01	
C22:2n6	0.02	0.02	0.02	0.02	0.02	0.01	
C23	0.04	0.06	0.04	0.02	0.04	0.04	
C24	0.02	0.02	0.03	0.04	0.02	0.02	
C22:6n3	2.14	0.89	2.78	1.14	1.83	0.75	
C24:1n9	0.23	0.71	0.03	0.03	0.02	0.01	
SFA	48.10	4.91	44.88	5.59	47.98	8.14	
MUFA	17.90	6.25	12.37	4.68	15.87	6.70	
PUFA	34.00	8.01	42.75	8.37	36.15	13.65	
□ MC-SFA	2.64	4.45	3.18	5.50	2.10	2.70	
□ LC-SFA	45.35	4.97	41.70	4.63	45.87	7.92	
□ PUFA n-3	7.74	4.31	9.40	4.06	7.49	6.83	
□ PUFA n-6	16.63	4.82	21.64	5.62	16.71	6.98	
n6/n3 ratio	3.72	3.19	2.91	2.18	3.95	2.65	

	Cholesterol	7a-OH	7b-OH	5,6b-Epoxy	5,6a-Epoxy	Triol	Tetro	Total Ctrys	7b-OHCtrys	5,6b-EpoxyCtrys	5,6a-EpoxyCtrys	TriolCtrys	TetroCtrys	7a-OHTbCtrys	5,6a-Epoxy/5,6b-Epoxy	C10	C11	C12	C14	6-Keto	C15	C14E45	C16	C16Ete7	C17	C18	C18:trans9	C18:1cis9	C18:2Cis6	C18:3n6	C18:3n3	C20	C20:1n9	C20:2n6	C20:3n6	C20:4n6	C20:5n3	C22	C22:1n9	C22:2n6	C23	C24	C22:6n3	C24:1n9	SFA	MUFA	PUFA	∑ MediumCSFA	∑ LongCSFA	∑ PUFAn3	∑ PUFAn6	n6/n3ratio
Cholesterol	1																																																			
7a-OH	.742**	1																																																	
7b-OH	.698**	.744**	1																																																
5,6b-Epoxy	-0.169	0.010	0.281	1																																															
5,6a-Epoxy	-0.234	-0.216	0.070	.935**	1																																														
Triol	-0.374	0.059	-0.052	0.389	0.384	1																																													
Tetro	-.481	-0.133	-0.074	0.323	0.317	.549**	1																																												
Total Ctrys	-0.229	0.142	0.231	.958**	.958**	.439	.842**	1																																											
7a-OHCtrys	.494*	0.084	-0.049	.544*	.509*	.642**	.817**	.767**	1																																										
7b-OHCtrys	0.350	-0.056	0.055	.502*	.473*	.640**	.796**	.713**	.964**	1																																									
5,6b-EpoxyCtrys	0.131	0.201	0.257	.741**	.634**	-0.020	-0.092	51.7	-0.184	-0.214	1																																								
5,6a-EpoxyCtrys	-0.205	-0.176	0.092	.876**	.924**	0.173	0.122	.757**	-0.383	-0.396	.561**	1																																							
TriolCtrys	-0.252	-.552**	-.585**	-0.172	-0.289	0.174	-0.142	-.466*	0.223	0.257	-.527*	-0.141	1																																						
TetroCtrys	-.478*	-0.283	-0.192	-0.098	-0.202	0.366	.767**	0.063	-.496*	-.449*	-0.440	-0.343	0.183	1																																					
7a-OHTb-OH	0.051	0.086	-.466*	-0.240	-0.227	.428*	0.112	-0.016	-0.087	-0.367	-0.156	-0.150	0.190	0.138	1																																				
5,6a/5,6b-Epoxy	0.450	0.331	0.133	-0.129	-.502*	-0.315	-0.305	-0.452	0.329	0.330	0.381	-.549*	-0.389	-0.119	0.022	1																																				
C10	0.250	0.259	0.277	-0.094	-0.024	-0.152	0.053	-0.045	0.067	0.039	-0.021	0.111	-0.328	0.196	-0.108	0.112	1																																				
C11	-0.201	-0.070	0.144	0.258	0.015	0.222	0.299	0.022	-0.252	-0.022	0.079	0.084	0.052	.447*	0.048	-0.025	-0.106	1																																			
C12	0.292	0.160	0.213	-0.175	-0.255	-0.212	0.031	-0.109	0.029	0.040	0.030	-0.359	-0.336	0.192	-0.103	.675**	.676**	-0.149	1																																			
C14	0.167	-0.145	-0.051	-.505*	-.528*	-0.282	-0.056	-.393*	0.122	0.135	-0.328	-.595**	0.078	0.278	-0.109	.469*	.421	-0.103	.804**	1																																
6-Keto	0.277	0.266	-0.584**	0.140	0.686**	0.518*	0.364	0.511	-0.455	-0.569	-0.553	0.561	0.561	0.373	0.742**	-0.651	0.370	.560*	-0.153	-0.263	1																															
C15	-0.201	-0.216	-0.195	-0.110	-0.258	-0.119	0.118	-0.205	-0.080	-0.019	-0.106	-0.258	0.239	0.255	-0.043	0.039	-0.340	0.275	-0.356	-0.149	0.022	1																														
C14:1n5	-0.189	-.588**	-0.328	-0.493	-0.447	-0.421	-0.346	-.518*	0.245	0.282	-0.390	-0.537	.561**	0.116	-0.341	-0.152	-0.399	-0.148	-0.120	.611**	-0.068	.518*	1																														
C16	-0.334	-0.130	-0.069	0.082	-0.317	0.276	0.282	-0.085	-0.276	-0.129	-0.053	-0.288	0.240	.435*	0.075	-0.003	-.402*	.748**	-0.356	-0.153	0.579**	.529**	-0.111	1																													
C16:1n7	-0.057	-0.329	-0.240	-0.320	-0.322	-0.117	-0.117	-0.263	0.070	0.172	-0.208	-0.221	.518*	0.104	-0.198	-0.029	-0.362	0.189	-0.260	0.204	-0.042	0.356	0.432*	.406*	1																											
C17	-0.178	-0.378	-0.255	-0.478	-0.316	-0.097	-0.248	-0.380	0.187	0.186	-.583*	-0.315	.477*	-0.040	-0.051	-0.309	-.494*	-0.085	-.459*	0.279	-0.087	0.263	.717**	0.003	.532*	1																										
C18	-0.171	-0.353	-0.029	-0.179	-0.216	0.210	0.045	-0.101	-0.057	-0.028	-0.379	-0.154	0.196	0.112	0.255	-0.260	-0.375	.583*	-.456*	-0.007	0.740	0.269	0.159	.464*	0.014	-.540*	1																									
C18:1trans9	-0.023	-.415*	-0.065	-0.437	-0.379	-0.254	-0.298	-.406*	0.181	0.241	-0.382	-0.398	0.378	-0.016	-0.261	0.003	-0.369	-0.089	-0.157	.440*	-0.734	0.281	.714**	0.128	.514**	.815**	0.253	1																									
C18:1cis9	-0.088	-0.192	-0.185	-0.281	-0.380	0.014	0.064	-0.294	-0.023	0.060	-0.220	-0.447*	.529*	0.369	-0.014	0.096	-0.243	0.339	-0.180	0.225	-0.466	0.338	0.254	.646*	.873**	0.300	0.144	.495*	1																								
C18:2Cis6	-0.251	-0.068	-0.188	0.194	0.324	0.087	0.293	.394*	-0.282	-0.333	0.168	0.251	-0.272	0.081	0.197	-0.081	0.057	-0.196	0.055	-.412*	-0.038	-0.055	-0.341	-0.203	-.467*	-.530*	-0.324	-.526*	-0.369	1																							
C18:3n6	-0.064	-0.068	-0.085	-0.178	-0.081	-0.227	-0.251	-0.135	0.144	0.104	0.254	-0.078	-0.261	-0.266	-0.012	0.305	-0.092	-0.129	-0.092	0.055	-0.525	-0.028	0.067	-0.172	0.059	0.244	-0.022	0.268	-0.112	-0.152	1																					
C18:3n3	0.301	0.242	0.188	0.234	0.289	-0.170	-0.249	0.173	0.142	0.000	0.314	0.387	-0.246	-.478*	-0.215	-0.131	0.253	-.626*	0.032	-0.110	-.998**	-0.318	-0.140	-.622*	-0.186	-0.173	-.641*	-0.080	-.459*	0.031	0.135	1																					
C20	-0.053	0.256	0.099	-0.029	-0.225	0.285	0.367	0.176	-0.346	-0.182	-0.130	-0.238	-0.170	0.319	0.385	0.032	-0.162	0.364	-0.144	-0.187	0.751	.426*	-0.186	.452*	-0.331	-0.029	.585*	-0.206	0.018	0.215	-0.262	-.583*	1																				
C20:1n9	-0.310	-0.057	-0.081	0.117	0.034	.457*	0.266	0.136	-0.288	-0.194	-0.129	0.077	0.288	0.216	0.294	-0.353	-.428*	.644**	-.501*	-0.265	0.865	0.290	-0.145	.704**	0.189	0.209	.775**	-0.039	.406*	-0.144	-0.225	-.727**	.618**	1																			
C20:2n6	-0.109	0.114	0.031	0.215	0.099	0.353	0.171	0.163	-0.092	-0.067	-0.016	0.170	0.020	0.023	0.357	-0.235	-0.190	0.300	-0.217	-0.364	0.660	0.035	-0.072	0.076	-.544*	-0.089	.543*	-0.329	-.409*	0.247	-0.232	-0.341	.569**	.517**	1																		
C20:3n6	0.017	0.013	-0.030	0.205	0.368	0.096	-0.162	0.099	0.194	0.106	0.027	.481*	-0.064	-0.344	0.138	-0.361	-0.028	-0.228	-0.228	-.500*	0.589	-0.230	-0.053	-.547*	-.603*	-0.075	-0.054	-0.384	-.780**	0.274	0.081	0.349	-0.069	-0.219	.484*	1																	
C20:4n6	0.027	0.096	0.083	0.381	.654**	0.071	-0.069	0.345	0.076	0.040	0.138	.647**	-0.293	-.463*	0.008	-0.448	-0.067	-0.330	-0.207	-.535*	0.502	-0.320	-0.231	-.583*	-.648**	-0.141	-0.034	-.445*	-.826**	0.317	0.062	0.325	-0.024	-0.147	.495*	.697**	1																	
C20:5n3	0.139	0.123	0.112	0.429	.703**	-0.197	-0.269	0.023	.404*	0.286	0.299	.793**	-0.171	-0.316	-0.035	-0.449	0.165	-0.233	-0.116	-.462	0.558	-0.333	-.557*	-.475	-.491	-0.301	-0.248	-.426	-.510**	0.288	0.011	.410*	-0.267	-0.361	0.186	.737**	.680**	1																	
C22	0.055	0.210	0.026	-0.061	-0.143	0.322	0.123	0.088	-0.154	-0.159	-0.136	-0.087	0.011	0.005	.549*	-0.088	-0.329	0.352	-0.328	-0.339	0.808	0.216	-0.163	0.260	-0.333	0.060	.703**	-0.168	-0.140	0.152	-0.111	-.511*	.804**	.696**	.779**	0.259	0.261	-0.072	1															
C22:1n9	-0.071	0.190	0.103	-0.097	-0.051	0.186	-0.155	0.058	-0.088	-0.059	-0.058	0.010	-0.044	-0.202	0.189	-0.123	-0.166	0.128	-0.242	-0.114	0.339	-0.032	0.006	0.027	-0.213	0.234	.574*	-0.054	-0.166	-0.041	0.117	-0.271	.384*	.537**	.593**	0.185	0.248	-0.152	.578**	1													
C22:2n6	-0.061	0.078	-0.113	-0.140	0.002	0.113	-0.014	0.096	-0.064	-0.059	0.069	0.038	-0.209	-0.193	0.384	-0.029	-0.106	-0.059	-0.265	-0.218	0.257	-0.043	-0.082	-0.215	-.389*	0.175	0.351	-0.140	-.394*	0.131	.612**	-0.073	0.252	0.173	.392*	.429*	.458*	0.132	.504**	.528**	1											
C23	-0.031	-0.037	-0.167	-0.183	-0.112	0.000	-0.093	-0.084	0.034	-0.044	0.116	-0.086	-0.152	-0.121	0.243	0.166	-0.070	-0.026	-0.207	0.004	-0.134	0.009	0.053	-0.147	-0.057	0.330	0.235	0.138	-0.148	-0.185	.977**	0.000	-0.030	-0.010	-0.004	0.215	0.167	0.011	0.177	0.253	.813**	1										
C24	0.009	0.171	-0.044	-0.142	-0.122	0.378	0.141	0.065	-0.165	-0.187	-0.201	-0.067	0.048	0.085	.618**	-0.137	-0.154	0.320	-0.212	-0.139	0.834	-0.054	-0.229	0.095	-0.284	0.146	.686**	-0.158	-0.126	0.082	-0.018	-.486	.633**	.697**	.746**	0.269	0.235	-0.088	.501**	.663**	.842**	0.320	1									
C22:6n3	0.132	0.155	0.080	0.193	0.409	-0.052	-0.263	0.088	0.364	0.222	0.072	0.407	-0.172	-.440*	0.060	-0.201	0.006	-0.312	-0.111	-.389*	0.483	-.414*	-0.339	-.565**	-.607**	-0.104	0.029	-.412*	-.713**	0.193	0.043	0.204	-0.031	-0.134	.432*	.617**	.876**	.793**	0.212	0.258	0.559	0.133	0.217	1									
C24:1n9	-0.027	-0.049	-0.085	-0.181	-0.162	-0.174	-0.222	-0.153	0.074	0.029	0.249	-0.134	-0.238	-0.221	0.026	0.313	-0.092	-0.106	-0.094	0.135	-0.512	0.058	0.087	-0.056	0.116	0.299	0.052	0.340	-0.006	-0.224	.978**	0.082	-0.170	-0.140	-0.242	-0.057	-0.095	-0.152	-0.071	0.152	.612**	.868**	0.017	-0.115	1								
SFA	-0.074	-0.019	0.077	-0.318	-.583*	0.059	0.202	-0.269	-0.155	-0.041	-0.295	-.586*	0.022	.519*	0.076	0.409	0.139	.630**	.395*	.580**	0.788	0.275	0.168	.985**	0.129	0.097	.493*	0.195	.406*	-0.340	-0.128	.894**	.433*	.413*	0.067	-.834**	-.651**	-.600**	0.203	0.103	-0.163	-0.074	0.211	-.519*	1								
MUFA	-0.095	-0.234	-0.208	-0.327	-0.384	-0.027	0.015	-0.319	0.001	0.085	-0.211	-0.455	.511*	0.321	-0.039	0.116	-0.285	0.305	-0.204	0.352	-0.662	0.347	0.365	.617**	.908**	0.379	0.153	.582**	.918**	-.416	-0.005	-.429	-0.025	0.372	-.446	-.769**	-.818**	-.609**	-0.165	-0.159	-0.345	-0.062	-0.146	-.715**	0.111	0.365	1						
PUFA	0.102	0.154	0.082	0.430	.724**	-0.018	-0.126	0.354	0.090	-0.028	0.338	.686**	-0.325	-.496	-0.022	-0.357	0.092	-.546*	-0.169	-.498*	-0.133	-0.374	-0.362	-.723**	-.631**	-0.269	-0.385	-.470	-.846**	.655**	0.070	.726**	-0.234	-.470	0.230	.614**	.931**	.726**	-0.019	0.034	0.308	0.082	-0.055	.716**	-0.068	-.926**	-.838**	1					
∑ MediumCSFA	0.299	0.193	0.229	-0.174	-0.247	-0.210	0.037	-0.109	0.031	0.038	0.027	-0.333	-0.345	0.203	-0.117	.633**	-.739**	-0.128	.677**	.608**	-0.134	-0.362	-0.171	-0.366	-0.279	-.425*	-.418*	-0.185	-0.189	0.055	-0.066	0.049	-0.146	.503**	-0.218	-0.218	-0.203	-0.094	-0.335	-0.240	-0.258	-0.200	-0.210	-0.100	-0.088	.588**	-0.216	-0.068	1				
∑ LongCSFA	-0.289	-0.158	-0.083	-0.183	-0.423	0.223	0.192	-0.186	-0.072	-0.329	-0.368	0.389	0.396	0.158	-0.054	-0.392	.691**	.548**	0.241	.803**	0.328	.594**	.803**	0.318	.557**	-0.379	-0.104	.753**	.559**	.796**	0.238	-.481	-.539**	-.542*	.455*	0.274	-0.008	0.010	0.368	-.452*	0.029	.747**	.548**	-.776**	0.024	-.798**	1					
∑ PUFAn3	0.309	0.258	0.193	0.365	0.349	-0.171	-0.268	0.177	0.208	0.045	0.329	0.447	-0.267	-.536**	-0.198	-0.181	0.239	-.652**	-0.006	-0.183	-0.857	-0.362	-0.174	-.687**	-0.266	-0.185	-.587**	0.169	-.574**	0.089	0.134	.848**	.555**	-.737**	-0.234	.886**	.481	.546**	-.438*	-0.256	0.002	0.029	-.414*	.598**	-.756**	-.547**	-.861**	1				
∑ PUFAn6	0.024	0.060	0.060	0.345	.614**	0.075	-0.108	0.300	0.102	0.054	0.125	.636**	-0.229	-.455*	0.036	-0.433	-0.064	-0.322	-0.217	-.536**	0.498	-0.307	-0.202	-.585**	-.649**	-0.124	-0.032	-.494*	-.832**	0.320	0.086	0.333	-0.033	-0.162	.494*	.612**	.947**	.793**	0.288	0.247	.475*	0.190	0.248	.896**	-0.068	-.695**	-.820**	.988**	-0.217	1			
n6/n3ratio	-0.311	-0.260	-0.151	-0.234	-0.222	0.115	0.259	-0.124	-0.137	0.044	-0.434	-0.293	0.208	.453*	0.166	-0.106	-0.340	.657**	-0.183	-0.072	.885**	.470*	0.245	.800**	0.093	0.194	.667**	0.139	0.296	0.085	-0.153	-.862**	.555**	.647**	.697**	-0.171	-0.177	-0.272	.574**	0.201	0.107	-0.005	.495*	-0.200	-0.123	.552**	0.275	-.495*	-0.198	.718**	-.849**	-0.177	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

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