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Genetic and environmental influences on serum oxylipins, endocannabinoids, bile acids and steroids

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ABSTRACT

Lipid bioactivity is a result of direct action and the action of lipid mediators including oxylipins, endocannabinoids, bile acids and steroids. Understanding the factors contributing to biological variation in lipid mediators may inform future approaches to understand and treat complex metabolic diseases. This research aims to determine the contribution of genetic and environmental influences on lipid mediators involved in the regulation of inflammation and energy metabolism. This study recruited 138 monozygotic (MZ) and dizygotic (DZ) twins aged 18–65 years and measured serum oxylipins, endocannabinoids, bile acids and steroids using liquid chromatography mass-spectrometry (LC-MS). In this classic twin design, the similarities and differences between MZ and DZ twins are modelled to estimate the contribution of genetic and environmental influences to variation in lipid mediators. Heritable lipid mediators included the 12-lipoxygenase products 12-hydroxyeicosatetraenoic acid [0.70 (95% CI: 0.12,0.82)], 12-hydroxyeicosatetraenoic acid [0.73 (95% CI: 0.30,0.83)] and 14-hydroxydocosahexaenoic acid [0.51 (95% CI: 0.07,0.71)], along with the endocannabinoid docosahexaenoylethanolamide [0.52 (95% CI: 0.15,0.72)]. For others such as 13-hydroxyoctadecatrienoic acid and lithocholic acid the contribution of environment to variation was stronger. With increased understanding of lipid mediator functions in health, it is important to understand the factors contributing to their variance. This study provides a comprehensive analysis of lipid mediators and extends pre-existing knowledge of the genetic and environmental influences on the human lipidome.

1. Introduction

Metabolomics is applied in nutrition and health research to characterize metabolic states, identify potential biomarkers, and monitor the effects of diet or drugs on metabolism. However, intra and inter-individual variation exists in metabolite concentrations which adds to the inherent complexity. Twin studies examine the contribution of genes and environment to variation in metabolite concentrations across different metabolomic platforms [1, 2]. Advances in analytical chemistry has improved the capacity to measure novel low abundance molecules [3]. Lipids have many structural, energetic and signalling functions which play important roles in health and disease. Lipid bioactivity is a result of their direct action and the action of metabolic by-products such as lipid mediators. Omega-6 (ω6) and omega-3 (ω3) poly-unsaturated fatty acids (PUFA) are substrates for enzymes that generate biologically active metabolites, including oxylipins and endocannabinoids. Whereas cholesterol is an essential precursor for the synthesis of bile acids (BA) and steroid hormones.

Oxylipins are oxygenated bioactive molecules regulating processes including inflammation, glucose metabolism and cardiovascular homoeostasis [4–6]. Oxylipins are synthesised through 4 pathways, the cyclooxygenase pathway (COX) producing prostaglandins and thromboxanes, the lipoxygenase pathway (LOX) producing hydroperoxy-PUFAs, the cytochrome p450 pathway (CYP) producing epoxides and ω-hydroxides, and the non-enzymatic pathway producing peroxide-associated products [4]. Endocannabinoids are FA derivatives synthesised from membrane phospholipid precursors with roles in

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Abbreviations

AA	arachidonic acid
AEA	n-arachidonoylethanolamine
ALA	alpha-linoleic acid
aLEA	alpha-linoleoylethanolamide
Auto-ox	auto-oxidation
BAT	bile acid transferase
CA	cholic acid
CDCA	chenodeoxycholic acid
COX	cyclooxygenase
CYP	cytochrome P450
Cyp27A1	Cytochrome P450 Family 27 Subfamily A Member 1
DCA	Deoxycholic acid
EPA	eicosapentaenoic acid
EPEA	eicosapentaenoyl ethanolamide
DHEA	docosahexaenoy-lethanolamide
DGLA	dihomo-g-linoleic acid
DHA	docosahexaenoic acid
DGLEA	dihomo-gamma-linolenoyl ethanolamide
FAAH	fatty-acid amide hydrolase
GCA	glycocholic acid
GDCA	glycodeoxycholic acid
GCDCA	glycochenodeoxycholic acid
GLCA	glycolithocholic acid
GUDCA	glycoursodeoxycholic acid
LA	linoleic acid
LCA	lithocholic acid
LEA	linoleoylethanolamide
LOX	lipoxygenase
LTB4	leukotriene B4
MCA	muricholic acid
NA-Gly	n-arachidonylglycine
NO-Gly	n-oleoylglycine
OA	oleic acid
OEA	N-oleoylethanolamide
PA	palmitic acid
PEA	palmitoylethanolamide
PGF2a	prostaglandin F2α
PLD	phospholipase D
POA	palmitoleic acid
POEA	palmitoleoyl ethanolamide
SA	stearic acid
SEA	steatroylethanolamide
sEH	soluble epoxide hydrolase
TCA	taurocholic acid
TCDCA	taurochenodeoxycholic acid
TDCA	taurodeoxycholic acid
TUDCA	tauroursodeoxycholic acid
TXB2	thromboxane B2
UDCA	ursodeoxycholic acid
11,12-DiHETrE	11,12-dihydroxyeicosatrienoic acid
11-HETE	11-hydroxyeicosatetraenoic acid
12,13-Ep-9-KODE	trans-12,13-epoxy-11-oxo-trans-9-octadecenoic acid
12,13-EpOME	12,13-epoxyoctadecamonoenoic acid
12,13-DiHOME	12,13-dihydroxyoctadeca(mono)enoic acid
12-HEPE	12-hydroxyeicosapentaenoic acid
12-HETE	12-hydroxyeicosatetraenoic acid
13-HODE	13-hydroxyoctadecadienoic acid
13-HOTE	13-hydroxyoctadecatrienoic acid
14,15-DiHETE	14,15-dihydroxyeicosatetraenoic acid
14,15-DiHETrE	14,15-dihydroxyeicosatrienoic acid
14-HDoHE	14-hydroxy-docosahexaenoic acid
15,16-EpODE	15,16-epoxyoctadecadienoic acid
15,16-DiHODE	15,16-dihydroxyoctadecadienoic acid
15-HETE	15-hydroxyeicosatetraenoic acid
17,18-DiHETE	17,18-dihydroxyeicosatetraenoic acid
19,20-DiHDoPA	19,20-dihydroxy-4,7,10,13,16-docosapentaenoic acid
5,6-DiHETrE	5,6-dihydroxyeicosatrienoic acid
5-HEPE	5-hydroxyeicosapentaenoic acid
5-HETE	5-hydroxyeicosatretraenoic acid
9,10-e-DiHO	9,10-dihydroxy-octadecenoic acid
9,10-EpO	9,10-epoxyoctadecanoic acid
9,10-EpOME	9,10-epoxyoctadecamonoenoic acid
9,10-DiHODE	9,10-dihydroxyoctadecadienoic acid
9,10-DiHOME	9,10-dihydroxyoctadeca(mono)enoic acid
9,12,13-TriHOME	9,12,13-trihydroxyoctadec-10-enoic acid
9-HODE	9-hydroxyoctadecadienoic acid
9-HOTE	9-hydroxyoctadecatrienoic acid
9-KODE	9-ketooctadecadienoic acid.

satiety, energy storage, nutrient transport, inflammation and insulin sensitivity [7]. Overstimulation of the endocannabinoid system results in increased levels of ω6 arachidonate-derived n-arachidonoyl-ethanolamide (AEA) and 2-arachidonylglycerol (2-AG) and is associated with obesity and diabetes [7]. The stimuli, cell type and abundance of parent FAs can influence the production of lipid mediators. High dietary ω3-PUFA intakes can increase tissue abundances of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and their associated lipid mediator derivatives at the expense of ω6-PUFA metabolites [5,8]. BAs regulate lipid, glucose and energy metabolism [9]. BAs facilitate digestion and absorption of nutrients and are reabsorbed from the gut and transported back to the liver to maintain the BA pool [9,10]. Steroids are synthesised in endocrine cells and regulate development, growth, reproduction and systemic homoeostasis [11]. Steroid biosynthetic pathways are regulated by cytochrome P450 enzymes or specialised hydroxysteroid dehydrogenase (HSD) enzymes [11]. Lipid mediators have important physiological functions in humans but substantial inter-individual variation exists in lipid pathways and in response to supplementation [4,12,13]. With important roles in health and disease states, improved understanding of the genetic and environmental factors contributing to lipid variation will maximise their application in research, diagnostics and treatment.

Twin studies exploit the commonalities and differences between twins to estimate the impact of genes and environment on variation in human traits [14]. Environmental factors including diet, drugs or sample collection, processing and handling are known to contribute to variation in lipid mediators [4]. In monozygotic (MZ) twins relative concentrations of LC-MS derived lipids are correlated and plasma lipidomic profiles cluster together strongly, highlighting the influence of genes [15,16]. In the TwinsUK cohort [1] heritability estimates reported for 503 blood metabolites included a small selection of BAs, steroids and one eicosanoid. Across twin studies several lipid mediators have been investigated however, a comprehensive coverage of oxylipins, endocannabinoids, BAs and steroids and their associated heritability estimates in a healthy cohort would be a beneficial resource. This study aims to determine the contribution of genes and environment to variation in lipid mediators involved in the regulation of inflammation and energy metabolism.

2. Materials and methods

2.1. Study design

The UCD twin study is a classic twin cohort including healthy, male and female MZ and same-sex dizygotic (DZ) twins aged 18–65 years living in Ireland. The study design, inclusion and exclusion criteria were described previously [17]. One-hundred and thirty-eight participants (69 twin pairs) attended UCD for a fasted blood draw and constitute the study population for this research. Ethical approval was obtained from the Human Research Ethics Committee in University College Dublin and all participants provided informed written consent (LS-13–44-OSullivan). All procedures were conducted in accordance with the principles expressed in the Declaration of Helsinki.

2.2. Dietary analysis

Dietary intake was assessed on 5 non-consecutive days, over a 2-month period, using the 24hr recall method based on the US Department of Agriculture Automated Multiple-Pass Method (USDA AMPM). The dietary recalls were conducted in person by trained investigators, and a photographic atlas was used to estimate portion size. Dietary intake was recorded on weekdays and weekend days. Food intake data was coded and entered into WISP version 3.0 (Tinuviel Software, Anglesey, UK) for analysis. WISP version 3.0 used data from the fifth and sixth edition of McCance and Widdowson's *"The Composition of Foods"* plus supplemental volumes. The nutrient composition dataset was modified to include new recipes and new food codes. All data was quality controlled for accuracy. Mean daily intakes were calculated using the repeated 24hr dietary recalls.

2.3. Biofluid and anthropometric collection

After an over-night fast venous blood samples were collected (10 mL serum tube and 4 mL lithium heparin). Serum tubes were left 30 min at room temperature to allow clotting. Samples were centrifuged at 1500 RCF for 15 min at 4 °C. The supernatant fluids were stored at −80 °C until analysis. Buccal swabs were collected for zygosity analysis and confirmed by 21 DNA markers (Genetic Testing Laboratories Inc. Brighton, U.K.). Height was measured to the nearest millimetre with Leicester portable height measure (Chasmores Ltd., U.K.) without shoes. Body-mass was measured in duplicate using a Tanita body composition analyser BC-420MA (Tanita Ltd., GB), and body composition was measured by air-displacement plethysmography (BOD-POD, Life Measurements Instruments, Concord, USA).

2.4. Clinical chemistry

Standard commercial kits were used to measure clinical chemistry markers according to manufacturer's instructions. Plasma glucose, total cholesterol, high-density lipoprotein cholesterol, c-reactive protein as well as serum triacylglycerol and non-esterified fatty acids (NEFA) were measured using the Randox clinical chemistry analyser (Randox Laboratories, Antrim, U.K). The Friedewald (1972) formula was used to calculate low-density lipoprotein cholesterol [18]. Enzyme-linked immunosorbent assays (ELISA) were used to measure serum insulin (Mercodia Insulin ELISA) and serum high molecular weight (HMW) adiponectin (R&D systems Human HMW Adiponectin/Acrp30).

2.5. Oxylipins, endocannabinoids, PUFAs, BAs and steroids

Serum oxylipins, endocannabinoids, PUFAs, BAs and steroids were isolated and collected using modifications of previously published protocols [19]. A National Institute of Standards and Technology (NIST) standard reference material (SRM 1950–Metabolites in Human Plasma) was used as a quality control sample, with two samples per batch (NIST, Gaithersburg, MD). In brief, serum and SRM aliquots were spiked with deuterated surrogates, with an anti-oxidant solution and the internal standards, 1-cyclohexyl ureido, 3-dodecanoic acid and 1-phenyl ureido 3-hexanoic acid (CUDA/PHAU) in isopropanol. They were mixed with methanol:acetonitrile (50:50) and the homogenate was centrifuged (10 min, 4 °C, 2000 g). The supernatant was collected, filtered at 1 μm through PVDF membranes, and stored at −20 °C until analysis. Analyses were completed within 48 h. During analysis, samples were held in a chilled auto-sampling module at 10°C. Analytes were separated using a Waters Acquity ultra-performance LC (UPLC; Waters, Milford, MA) on a 2.1 mm × 150 mm, 1.7 μm BEHC 18 column (Waters) for oxylipin, endocannabinoid and PUFA analyses, and a 2.1 mm × 150 mm, 1.7 μm BEH C8 column (Waters) for BA and steroid analysis. Separated analytes were detected by tandem MS using electrospray ionization with multi reaction monitoring on an API 6500 QTRAP (Sciex, Redwood City, CA) for oxylipins, endocannabinoids, BAs and steroids. Analytes were quantified (nM) using internal standard methods and 7–9 point calibration curves of authentic standards. A complete list of sample identifiers, estimated limit of detection (LOD) and limit of quantification (LOQ) for the entire analytical target list, UPLC-MS/MS parameters and calibration standard concentration ranges are included in Supplementary Tables S1–11. Lipid mediators are unstimulated levels except for component involved in clotting.

2.6. Statistical analysis

Data are reported for analytes that had <30% missing values. Statistical analysis was carried out in Jmp v 14.0 (SAS Institute, Cary, NC) and R-statistical suite (version 3.6.1). Post-acquisition batch correction was performed using the SRM standards and the equation: $x_c = x_u + \tilde{n} - (b * m_b + Y_b)$, where x_c and x_u are the corrected and uncorrected concentrations for metabolite x respectively. $\tilde{n}$ is the average concentration of the metabolite in the SRM standard across all batches and b is the batch number. m and Y are the slope and intercept which can be obtained by linear regression with x-coordinates ($\tilde{n}$, average NIST standard for the batch) and y-coordinates (0, b). The resulting metabolite concentrations were Johnson-transformed and normality was confirmed using the Shapiro-Wilk test. Lipid pathways were examined by summing the oxylipins or endocannabinoids from the same pathway within an individual.

Univariate twin structural equation modelling (SEM) was performed on all metabolites, implemented in R-Package OpenMx, (version 2.10.0), controlling for age, gender and body-mass [20]. Using twin data to establish regularity and randomness of sampling, means and variances were examined to ensure equality across twin order and zygosity groups (Supplementary Table S12). SEM for the classic twin design models the covariance structure in phenotypic data obtained from MZ and DZ twins, decomposing variance into genetic, shared environmental and unique environmental variance and covariance components. A cohort of 69 MZ and DZ twin pairs is powered to estimate additive genetic effects (A) ≥ 76% with 80% power [21]. While this study is powered to estimate heritability for certain traits, the sample size is small. The full ACE model was preferred with no nested models presented.

A familial component, combining the genetic and shared environmental variance components, modelled familial variation with good precision for all lipid mediators. It is calculated as the proportion of total variance accounted by genetic and shared environmental effects ($V_A + V_C/V_A + V_C + V_E$). Due to small sample size, statistical power is low to detect low heritability or shared environment estimates. Thus combining the two factors and not further decomposing variance into genetic and shared environment is a conservative estimate of maximal heritability or familial effects. The circular stacked bar plot was created using R-package ggplot2.

3. Results

3.1. Characteristics of the cohort

The UCD Twin Study cohort includes 45 MZ and 24 DZ twins pairs, with 60 males and 78 females (Table 1). Participants had a mean age of 36 years (±13) and BMI of 24 kg/m^2 (±3) For the clinical chemistry measurements mean fasting glucose was 4.6 mmol/L (±0.4) and total-cholesterol was 5.1 mmol/L (±1.1). The contribution of protein, carbohydrate and fat to total energy were 17% (±4), 42% (±9) and 40% (±8) respectively.

3.2. Oxylipins, endocannabinoids, BAs and steroids

Serum oxylipins and endocannabinoids derived from FAs are presented in Table 2. ω6 derived oxylipins make up the largest proportions of the measured PUFA oxylipin pool (63%). LA-derived oxylipins account for the largest amount of oxylipins and within them 13-hydroxyoctadecadienoic acid (13-HODE) and 9-HODE, had the highest mean concentrations of 15.36 nM (±9.37) and 9.48 nM (±5.04) respectively. For AA-derived serum oxylipins, 12-hydroxyeicosatretraenoic acid (12-HETE) had the highest concentrations of 13.10 nM (±20.98), followed by thromboxane B2 (TXB2) with concentrations of 5.67 nM (±8.97). Soluble epoxide hydrolase (sEH) derived oxylipins had the highest concentrations for both ALA-derived oxylipin (i.e. 15,16-DiHODE) and EPA-derived oxylipins (i.e. 17,18-DiHETE and 14,15-DiHETE). BAs and steroids are also presented in Table 2. Primary BA (CA, CDCA) and conjugated-primary BA (GCA, TCA, GCDCA, TCDCA) concentrations for the total cohort ranged from 44.31 to 46.46 nM and 4.63–113.62 nM respectively. Cortisol had the highest concentrations of 75.49 nM (±33.30) for the measured steroids.

Table 1
Mean anthropometric, clinical chemistry and dietary intakes.

		Total	MZ	DZ
Gender	Males (n)	60	38	22
	Females (n)	78	52	26
Age	years	36 (±13)	36 (±12)	34 (±15)
Height	cm	169.9 (±8.7)	169.5 (±8.0)	170.6 (±9.9)
Waist	cm	79.9 (±8.8)	80.0 (±9.7)	79.9 (±7.1)
Hip	cm	98.0 (±7.7)	98.0 (±8.2)	98.1 (±6.7)
Body fat	%	26 (±10)	26 (±10)	26 (±10)
Weight	kg	70.2 (±11.5)	69.8 (±12.3)	70.9 (±10.0)
BMI	kg/m^2	24 (±3)	24 (±4)	24 (±3)
Total-cholesterol	mmol/L	5.1 (±1.1)	5.3 (±1.1)	4.8 (±1.0)
HDL-cholesterol	mmol/L	1.7 (±0.4)	1.8 (±0.5)	1.7 (±0.4)
LDL-cholesterol	mmol/L	3.0 (±0.9)	3.1 (±0.9)	2.8 (±0.9)
Triacylglycerol	mmol/L	0.9 (±0.4)	0.9 (±0.5)	0.8 (±0.4)
NEFA	mmol/L	0.7 (±0.3)	0.8 (±0.3)	0.7 (±0.3)
Glucose	mmol/L	4.6 (±0.4)	4.5 (±0.4)	4.7 (±0.3)
CRP	mg/dl	1.1 (±1.9)	1.1 (±2.2)	1.0 (±1.2)
Insulin	mu/L	4.1 (±2.5)	3.7 (±1.9)	5.0 (±3.2)
Adiponectin	ug/mL	4.4 (±2.8)	4.7 (±3.1)	3.7 (±2.1)
Energy	kcal	2060 (±610)	2050 (±649)	2081 (±537)
Protein	g	87.7 (±34.0)	87.7 (±36.9)	87.7 (±28.1)
Carbohydrate	g	231.5 (±79.2)	228.6 (±84.1)	237.0 (±69.5)
Fat	g	92.9 (±34.5)	93.0 (±37.7)	92.8 (±27.8)
SFA	g	34.5 (±14.6)	34.9 (±16.3)	33.6 (±10.9)
MUFA	g	31.2 (±12.2)	31.2 (±12.5)	31.3 (±11.7)
PUFA	g	17.3 (±7.4)	17.2 (±7.9)	17.5 (±6.5)
% protein to total energy	%	17 (±4)	17 (±4)	17 (±3)
% CHO to total energy	%	42 (±9)	42 (±10)	43 (±67)
% fat to total energy	%	40 (±8)	40 (±9)	40 (±6)

Mean and standard deviations are presented. MZ: monozygotic; DZ: dizygotic; BMI: body-mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA: non-esterified fatty-acids; CRP: c-reactive protein; SFA: saturated fatty-acids; MUFA: mono-unsaturated fatty-acids; PUFA: poly-unsaturated fatty-acids derived from dietary recall data; CHO: carbohydrate; cm: centimetres;%: percentage; kg: kilograms; m2: meters squared; mmol/L: millimole Per Litre: litre; mu/L: Milliunits Per Litre; ug/L: microgram Per Litre; kcal: kilo-calories; g: gram.

3.3. Heritability estimates for oxylipins, endocannabinoids, BAs and steroids

Genetic, shared environmental and unique environmental estimates for oxylipins, endocannabinoids, BAs and steroids are presented in Fig. 1, grouped according to their primary biosynthetic pathways. Full ACE estimates and confidence intervals are presented in Supplementary Table S13. 12-LOX dependant oxylipins were the most heritable. For the AA-derived oxylipin 12-HETE, the heritability estimates were 0.70 (95% CI: 0.14, 0.84) and the unique environmental estimate was 0.26 (95% CI: 0.16, 0.43). Similarly, 12-HEPE, an EPA derived oxylipin produced through the 12-LOX pathway, had a high heritability estimate of 0.74 (95% CI: 0.32, 0.84) and a unique environmental estimate of 0.26 (95% CI: 0.16, 0.43). 14-hydroxy-docosahexaenoic acid (14-HDoHE) a DHA-derived 12-LOX metabolite had a heritability estimate of 0.52 (95% CI: 0.07, 0.71) and unique environmental estimate of 0.48 (95% CI: 0.29, 0.76). The ω3 endocannabinoid docosahexaenoy-lethanolamide (DHEA) had the highest heritability of the endocannabinoids, with a heritability estimate of 0.56 (95% CI: 0.19, 0.75). Heritability estimates for the BAs, regulated by cytochrome P450 enzymes (CYP27A1 gene), ranged from 0.04 to 0.53. Whereas BAs influenced by the microbiome had lower heritability estimates ranging from 0.00 to 0.28. For the steroids, heritability estimates ranged from 0.18 to 0.30, apart from corticosterone which was influenced by shared environmental effects [0.40 (95% CI: 0.00,0.58)]. Variance explained by a familial factor which combined the genetic and shared environmental effects was also estimated for all the lipid mediators (Table 2). Four analytes were solely influenced by unique environmental effects, these include 5,6-DiHETrE, 13-HOTE, aLEA and LCA. For all the other analytes familial factors accounted for 0.08–0.74 of variance; with higher familiality suggesting similarities in concentrations between twins.

3.4. Familial influences on lipid pathways

Familial and unique environmental estimates were modelled for the lipid pathways. Familial estimates for the 14 pathways are presented in Fig. 2. Familial estimates ranged from 0.06 to 0.75. An analyte pathway regulated by the 12-LOX enzyme had the largest significant familial component estimate of 0.75 (95% CI: 0.59, 0.85), with the remainder of variance explained by a unique environment factor [0.25 (95% CI: 0.15, 0.41)]. Pathways regulated by COX-1 and PLD also had high familial estimates of 0.55 (95% CI: 0.34, 0.71) and 0.52 (95% CI: 0.21, 0.73) respectively.

4. Discussion

A wide range of heritability estimates are reported for 66 profiled lipid mediators. Heritable lipid mediators included 12-HETE, 12-HEPE, 14-HDoHE and DHEA. The contribution of environmental influences to variance was stronger for other analytes. This study also demonstrates that lipid pathways regulated by 12-LOX, COX-1 and PLD enzymes were more strongly influenced by familial factors compared to other pathways. This is one of few twin studies to report the contribution of genetic factors to variance in many of these lipid mediators. Thus this study adds important information to pre-existing knowledge and decomposes variance in serum lipid mediators into genetic, shared and unique

Table 2
Mean (nM) concentrations and modelling estimates of serum oxylipins, endocannabinoids BA and steroids.

Metabolite	Pathway	Total (n = 128)	Familial factor	Unique environmental factor
LA-derived oxylipin				
9,12,13-TriHOME	Auto-ox	1.78 (±1.11)	0.31 (0.05, 0.57)	0.69 (0.43, 0.95)
12,13-DiHOME	sEH	7.29 (±4.45)	0.37 (0.12, 0.58)	0.63 (0.42, 0.88)
9,10-DiHOME	sEH	7.12 (±5.06)	0.31 (0.05, 0.54)	0.69 (0.46, 0.95)
13-HODE	Auto-ox/15-LOX1/COX	15.36 (±9.37)	0.45 (0.20, 0.64)	0.55 (0.36, 0.80)
9-HODE	Auto-ox/LOX	9.48 (±5.04)	0.30 (0.02, 0.53)	0.70 (0.47, 0.98)
9-KODE	ADH	1.77 (±1.03)	0.14 (0.00, 0.40)	0.86 (0.60, 1.00)
12,13-Ep-9-KODE	ADH	3.53 (±4.82)	0.36 (0.09, 0.57)	0.64 (0.43, 0.91)
12,13,-EpOME	CYP	3.87 (±2.60)	0.40 (0.16, 0.60)	0.60 (0.40, 0.84)
9,10-EpOME	CYP	1.25 (±0.87)	0.12 (0.00, 0.36)	0.88 (0.64, 1.00)
LA-derived endo				
LEA	PLD	4.93 (±1.45)	0.32 (0.00, 0.59)	0.68 (0.41, 1.00)
DGLA-derived endo				
DGLEA	PLD	0.15 (±0.13)	0.27 (0.00, 0.51)	0.73 (0.49, 1.00)
AA-derived oxylipins				
TXB2	COX1	5.67 (±8.97)	0.54 (0.32, 0.70)	0.46 (0.30, 0.68)
PGF2a	COX2	0.30 (±0.20)	0.09 (0.00, 0.32)	0.91 (0.68, 1.00)
LTB4	5-LOX	0.36 (±0.34)	0.43 (0.17, 0.63)	0.57 (0.37, 0.83)
14,15-DiHETrE	sEH	1.01 (±0.34)	0.22 (0.00, 0.48)	0.78 (0.52, 1.00)
11,12-DiHETrE	sEH	0.96 (±0.33)	0.28 (0.02, 0.53)	0.72 (0.47, 0.98)
5,6-DiHETrE	CYP	0.49 (±0.21)	0.00 (0.00, 0.27)	1.00 (0.73, 1.00)
15-HETE	15-LOX	1.28 (±0.80)	0.31 (0.06, 0.53)	0.69 (0.47, 0.94)
12-HETE	12-LOX	13.10 (±20.98)	0.74 (0.57, 0.84)	0.26 (0.16, 0.43)
11-HETE	COX	0.80 (±0.61)	0.45 (0.24, 0.65)	0.55 (0.35, 0.76)
5-HETE	5-LOX	1.80 (±1.27)	0.33 (0.06, 0.55)	0.67 (0.45, 0.94)
AA-derived endo				
AEA	PLD	2.60 (±0.85)	0.46 (0.19, 0.67)	0.54 (0.33, 0.81)
NA-Gly	FAAH	0.63 (±0.74)	0.44 (0.16, 0.64)	0.56 (0.36, 0.84)
ALA-derived oxylipins				
15,16-DiHODE	sEH	16.09 (±11.77)	0.09 (0.00, 0.35)	0.91 (0.65, 1.00)
9,10-DiHODE	sEH	0.32 (±0.27)	0.31 (0.05, 0.54)	0.69 (0.46, 0.95)
13-HOTE	Auto-ox/15-LOX1/COX	1.39 (±0.79)	0.00 (0.00, 0.20)	1.00 (0.80, 1.00)
9-HOTE	Auto-ox/LOX	0.53 (±0.44)	0.14 (0.00, 0.39)	0.86 (0.61, 1.00)
15,16-EpODE	CYP	4.29 (±2.71)	0.01 (0.00, 0.26)	0.99 (0.74, 1.00)
ALA-derived endo				
aLEA	PLD	0.27 (±0.11)	0.00 (0.00, 0.23)	1.00 (0.77, 1.00)
EPA-derived oxylipins				
14,15-DiHETE	sEH	1.15 (±0.70)	0.08 (0.00, 0.34)	0.92 (0.66, 1.00)
17,18-DiHETE	sEH	8.29 (±4.34)	0.19 (0.00, 0.43)	0.81 (0.57, 1.00)
12-HEPE	12-LOX	0.59 (±0.84)	0.74 (0.57, 0.84)	0.26 (0.16, 0.43)
5-HEPE	5-LOX	1.03 (±0.64)	0.21 (0.00, 0.48)	0.79 (0.52, 1.00)
EPA-derived endo				
EPEA Screen	PLD	0.05 (±0.03)	0.36 (0.10, 0.58)	0.64 (0.42, 0.90)
DHA-derived oxylipin				
19,20-DiHDoPA	sEH	2.62 (±1.24)	0.25 (0.00, 0.50)	0.75 (0.50, 1.00)
14-HDoHE	12-LOX	2.64 (±3.12)	0.52 (0.24, 0.71)	0.48 (0.29, 0.76)
DHA-derived endo				
DHEA	PLD	2.07 (±0.87)	0.56 (0.27, 0.75)	0.44 (0.25, 0.73)
OA-derived oxylipin				
9,10-e-DiHO	sEH	7.45 (±4.00)	0.13 (0.00, 0.38)	0.87 (0.62, 1.00)
9,10-EpO	CYP	2.31 (±2.67)	0.12 (0.00, 0.40)	0.88 (0.60, 1.00)
OA-derived endo				
OEA	PLD	9.09 (±2.90)	0.35 (0.01, 0.62)	0.65 (0.38, 0.99)
NO-Gly	FAAH	5.12 (±2.78)	0.26 (0.00, 0.52)	0.74 (0.48, 1.00)
PA-derived oxylipin				
PEA	PLD	5.17 (±2.31)	0.39 (0.13, 0.60)	0.61 (0.40, 0.87)
POA-derived endo				
POEA Screen	PLD	0.30 (±0.20)	0.31 (0.00, 0.56)	0.69 (0.44, 1.00)
SA-derived oxylipin				
SEA	PLD	1.58 (±1.31)	0.34 (0.03, 0.60)	0.66 (0.40, 0.97)
Bile Acids				
CA	Cyp27A1	46.46 (±97.88)	0.36 (0.08, 0.58)	0.64 (0.42, 0.92)
CDCA	Cyp27A1	44.31 (±66.27)	0.27 (0.00, 0.54)	0.73 (0.46, 1.00)
MCA	Cyp27A1	2.04 (±2.43)	0.53 (0.29, 0.70)	0.47 (0.30, 0.71)
α-MCA	Cyp27A1	0.33 (±0.22)	0.04 (0.00, 0.32)	0.96 (0.68, 1.00)
β-MCA	Cyp27A1	0.54 (±0.44)	0.37 (0.12, 0.58)	0.63 (0.42, 0.88)
GCA	Cyp27A1 + BAT	4.63 (±7.27)	0.38 (0.13, 0.59)	0.62 (0.41, 0.87)
TCA	Cyp27A1 + BAT	5.08 (±11.80)	0.30 (0.07, 0.53)	0.70 (0.47, 0.93)
GCDCA	Cyp27A1 + BAT	113.62 (±144.18)	0.35 (0.08, 0.56)	0.65 (0.44, 0.92)
TCDCA	Cyp27A1 + BAT	55.58 (±76.97)	0.33 (0.06, 0.55)	0.67 (0.45, 0.94)
Tα-MCA	Cyp27A1 + BAT	0.87 (±1.16)	0.20 (0.00, 0.46)	0.80 (0.54, 1.00)
GDCA	Microbiome + BAT	44.01 (±54.88)	0.26 (0.00, 0.49)	0.74 (0.51, 1.00)
TDCA	Microbiome + BAT	5.75 (±9.91)	0.27 (0.00, 0.50)	0.73 (0.50, 1.00)
GLCA	Microbiome + BAT	11.44 (±11.32)	0.15 (0.00, 0.39)	0.85 (0.61, 1.00)

(*continued on next page*)

Table 2 (*continued*)

Metabolite	Pathway	Total (n = 128)	Familial factor	Unique environmental factor
TUDCA	Microbiome + BAT	6.81 (±4.27)	0.12 (0.00, 0.36)	0.88 (0.64, 1.00)
GUDCA	Microbiome + BAT	14.69 (±20.91)	0.28 (0.01, 0.52)	0.72 (0.48, 0.99)
DCA	Microbiome	77.96 (±68.28)	0.33 (0.11, 0.57)	0.67 (0.43, 0.89)
LCA	Microbiome	12.19 (±8.31)	0.00 (0.00, 0.20)	1.00 (0.80, 1.00)
UDCA	Microbiome	58.48 (±69.37)	0.19 (0.00, 0.44)	0.81 (0.56, 1.00)
Steroids				
Corticosterone	Cyp11B1	1.98 (±1.95)	0.40 (0.18, 0.62)	0.60 (0.38, 0.82)
Cortexolone	Cyp21A1	0.15 (±0.10)	0.43 (0.19, 0.63)	0.57 (0.37, 0.81)
Cortisol	CyP11B1	75.49 (±33.30)	0.34 (0.07, 0.57)	0.66 (0.43, 0.93)
Cortisone	HSD21B1	15.67 (±3.71)	0.30 (0.03, 0.53)	0.70 (0.47, 0.97)

Mean (nM) and standard deviations are presented. Univariate ACE modelling was controlled for age, gender and body mass. Variance estimates and 95% CI are presented. Analytes have been Johnson transformed and standardised. MZ: monozygotic; DZ: dizygotic; Auto-ox: auto-oxidation; sEH: soluble epoxide hydrolase; LOX: lipoxygenase; COX: cyclooxygenase; PLD: phospholipase D; FAAH: fatty-acid amide hydrolase; CYP: cytochrome P450; Cyp27A1: Cytochrome P450 Family 27 Subfamily A Member 1; BAT: bile acid transferase.

environmental components.

The results demonstrate a wide range of heritability estimates (0–74%) for profiled serum lipid mediators. Similarly, TwinsUK reported varied estimates for steroids and BAs [1]. McGurk et al. [22] reported heritability estimates ranging 45–82% for 11 plasma N-acylethanolamide (NAE) analytes in 196 families. In this study lower heritability estimates are reported for 7 NAEs (AEA, PEA, OEA, DHEA, SEA, LEA, POEA), possibly due to different biofluids and modelling methodology. A study of MZ twins discordant for schizophrenia reported dominant familial effects on AEA concentrations [23]. Similarly, in this study when genetic and shared environmental influences are combined, a familial factor contributes the largest proportion of variation for AEA and the majority of NAEs. With improved understanding of lipid mediator functions in health and disease, knowledge of whether genes or environment are dominant factors influencing variance could inform future interventions for metabolic disease. A recent study reported high

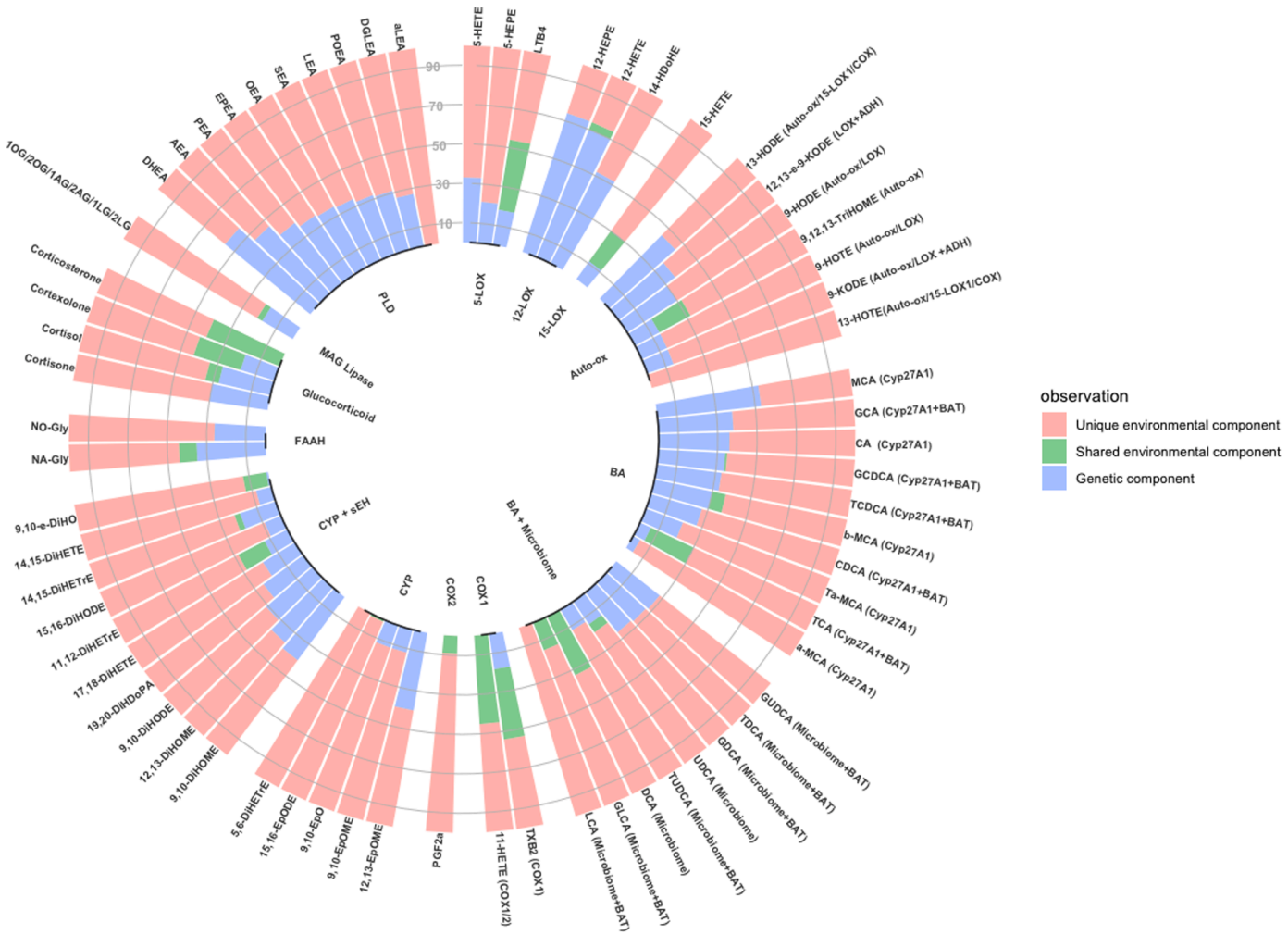

Fig. 1. ACE modelling estimates for oxylipins, endocannabinoids, BAs and steroids. Analytes are grouped according to pathways and heritability estimates are ranked highest to lowest within each group. LOX: lipoxygenase; Auto-ox: auto-oxidation; BA: Bile acids; COX: cyclooxygenase; CYP: cytochrome p450; sEH: soluble epoxide hydrolase; FAAH: fatty-acid amide hydrolase; MAG: monoacylglycerol; PLD: phospholipase D.

similarity between plasma and serum for many of these lipid mediators, suggesting generalizability of estimates to the blood compartment [24]. To date, few studies report the contribution of genetic and environmental influences to variance in the wide array of serum lipid mediators presented in this study.

Many lipid mediators were strongly influenced by unique environment, meaning analyte concentrations were not correlated within twins. Several factors including availability of PUFA precursors, the relative abundance and activity of specific enzymes, oxidative stress levels and health status of a person during sample collection affects lipid mediator concentrations [4]. The amount and type of dietary PUFAs will impact circulating levels of their lipid mediators [4]. Red blood cell membrane concentration, also known as the ω3 index, can be used as a stable biomarker of dietary intake detecting nutritional status more precisely than dietary assessment and is also a valid surrogate of ω3 content in tissues [25]. In Ireland, EPA and DHA intakes of adults aged 18–35 years are below the European Food Safety Authority recommended levels, most likely due to low fish intakes and fish oil supplementation practices [26]. Long chain ω3-PUFA supplementation can increase membrane concentrations of ω3-PUFA and derived lipid mediators at the expense of ω6 [6,27]. Importantly, many ω3-PUFA derived lipid mediators have anti-inflammatory and pro-resolving effects [28]. Therefore, public health approaches to improve dietary intakes of ω3-PUFA could elevate EPA and DHA lipid mediators playing an important role in mediating the cardioprotective and anti-inflammatory effects of EPA and DHA.

In this cohort 12-LOX derived oxylipins were the most heritable lipid mediators (0.49–0.73). Both ω3 and ω6 derived oxylipins were heritable, suggesting genes and gene expression impact the concentrations of these lipids. Considering that this study focused on serum as its primary matrix, the current study was well designed to identify additive genetic factors influencing lipid mediator involvement in clotting cascades. 12-LOX and its bioactive metabolites have important roles in platelet function, including dense granule secretion, normal aggregation and adhesion in platelets, making them potential targets in anti-platelet therapy with implications for cardiovascular disease [29,30]. Recently, age and obesity related lower plasma concentrations of 12-LOX oxylipins, 12-HETE, 12-HEPE and 14-HDoHE were associated with arterial stiffness, and higher risk of cardiovascular disease [31]. Twin and family studies support a genetic influence on platelet aggregation and cardiovascular disease [32,33]. Within the platelet activation process, studies focused on the pro-thrombotic COX-1-derived eicosanoid TXA2 have reported high heritability [34]. In this study, its degradation product TXB2 had a large familial component. The platelet phenotypic response to aspirin, a COX-1 inhibitor, is also heritable [35]. However, despite successful TXA2 inhibition by aspirin a significant portion of individuals continue to experience thrombotic events, a phenomenon termed anti-platelet resistance. 12-LOX mediated synthesis of 12-HETE is also inhibited by aspirin and may be a putative marker of aspirin response as variation in efficacy on 12-HETE inhibition is closely associated with the extent of inhibition of platelet aggregation [36]. This study shows 12-HETE is highly heritable in agreement with estimates reported in the TwinsUK cohort [1]. Shin et al. [1] also identified two SNPs (rs2271316 and rs2246200) that explained ~10% of the variance in 12-HETE. For this highly heritable trait, future GWAS could determine whether genotype has implications in anti-platelet therapy. Targeting 12-LOX represents a new approach for treating pathologies its derived oxylipins play a role in, such as thrombosis, inflammation, hypertension and type-1 diabetes mellitus. Future work must improve our understanding of how 12-LOX derived oxylipins are mechanistically regulating platelet reactivity, thrombosis, and other processes.

DHEA was the most heritable NAE. DHEA is ω3 derived with important roles in normal brain functioning and inflammatory processes [37,38]. Endogenous conversion of ALA to EPA and DHA is low in humans (~1%), and circulating DHA is predominantly from consuming food sources like oily fish, fish oils and shellfish [39,40]. The heritability of fish intake and dietary EPA and DHA intakes ranged 0.13–0.24 and 0.12–0.22 respectively across 17 European cohorts [41]. Whereas, circulating blood levels of DHA were more heritable at ~32% [1]. In this study, DHEA was heritable (~50%) and higher than estimates reported for its PUFA precursor DHA which may be explained by its dependence on pathways for synthesis. Environmental factors including income, education and other geographical, cultural and social characteristics, all related to ω3 intakes, may be modifiable targets to improve DHA and DHEA levels. The membrane enzyme N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) is a

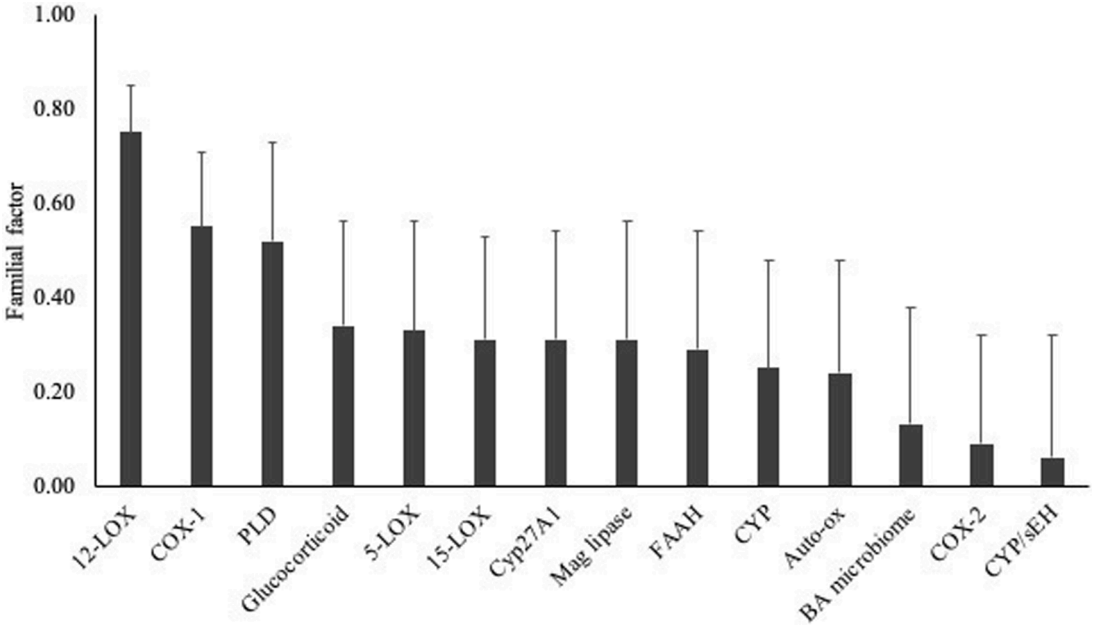

Fig. 2. Familial modelling estimates and 95% confidence interval for lipid pathways. LOX: lipoxygenase; COX: cyclooxygenase; PLD: phospholipase D, Cyp27A1: Cytochrome P450 Family 27 Subfamily A Member 1; MAG: monoacylglycerol; FAAH: fatty-acid amide hydrolase; CYP: cytochrome p450; Auto-ox: auto-oxidation; BA: Bile acids; sEH: soluble epoxide hydrolase1.

principal NAE biosynthetic enzyme. In humans, a common haplotype in NAPE-PLD (rs17605251) was protective against severe obesity showing the potential role for NAPE-PLD in human obesity through energy regulation [42]. However, NAPE-PLD inhibitors and NAPE-PLD knockout mice lack effect on ω3 NAEs suggesting these signalling lipids are partly produced through other pathways that are not fully understood [43,44]. Combining ω3 NAEs and GWAS could provide further insight into the genes and pathways influencing these traits which may have implications in the treatment of pain, satiety, inflammation and emotional states linked with this important family of signalling lipids.

Heritability estimates for the BAs ranged from 0 to 54% and environmental factors contributed the remainder of variance. Shin et al. [1] reported a selection of BAs (CA, GCA, TCA, GCDCA, TCDCA, DCA, GDCA, TDCA, UDCA, TUDCA and GUDCA) measured using UPLC-MS/MS in fasting blood samples. Heritability estimates for their BAs fell within 95% confidence intervals of the familial estimates reported in this study. The BA pool cycles between the liver and intestine. Results presented here suggest that primary BAs are more heritable than those dependant on the microbiome. This is unsurprising considering the microbiome is strongly environmentally driven [45]. The microbiome controls the composition of the bile acid pool through deconjugation and de-hydroxylation of primary BAs into secondary BAs [46,47]. Manipulating the microbiome through probiotic supplementation [48,49] or changing aspects of diet, such as high or low fat diets can impact BA synthesis and metabolism [50]. While postprandial bile acid metabolism is somewhat heritable [51], our findings show unique environmental influences explain the largest proportion of variance in fasting BAs. BAs have important roles in lipid, glucose and energy metabolism and are linked with multiple metabolic disorders [52]. In Alzheimer's disease a significant increase in secondary BAs via enzymatic activities in the gut microbiome are significantly correlated with poorer cognition and may contribute to the disease [53]. Future research examining the synergistic effects of dietary fat and the microbiome on BAs could provide insights into their application in metabolic disorders such as diabetes mellitus and Alzheimer's disease [53,54].

Strengths and limitations should be considered when interpreting the results. Strengths include the nature of this classic twin cohort which permits the analysis of genetic and environmental factors influencing variance in traits. The targeted LC-MS approach has improved accuracy and precision with better selectivity of low abundance lipid mediators. However, our cohort is healthy, and the sample is small relative to other twin cohorts. To account for some limitations, we ensured twin assumptions were not violated, incorporated covariates in all models and modelled a familial component alongside the full ACE model. Still sample numbers must be considered when interpreting model estimates. We acknowledge this research is exploratory and validating results using larger numbers would strengthen our findings.

In conclusion, this classic twin study shows that the contribution of genes and environment to variation in lipid mediators depends on the analyte and associated metabolic pathway. 12-LOX derived oxylipins are heritable, whereas oxylipins and BAs connected with cytochrome P450 enzymes are less so and more strongly influenced by unique environment. These observations have important research and potential clinical implications. For example, the stronger genetic contribution to 12-LOX derived oxylipins might impact cardiovascular disease risk or potentially explain response to drug treatments. Conversely, where unique environment is more dominant offers opportunities to identify new targets for intervention; for example, it might be possible to change concentrations of certain BAs by manipulating the microbiome. This is an exciting area of research that could potentially lead to novel metabolic disease treatments. However, more research is needed to better understand these important regulatory lipids and then test the potential derived interventions to improve metabolic health.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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CRediT authorship contribution statement

K.M. Bermingham: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Visualization, Writing – review & editing. **L. Brennan:** Conceptualization, Funding acquisition, Investigation, Investigation, Methodology, Software, Supervision, Visualization, Writing – review & editing. **R. Segurado:** Conceptualization, Formal analysis, Methodology, Supervision, Visualization, Writing – review & editing. **I.J. Gray:** Data curation, Investigation, Methodology, Writing – review & editing. **R.E. Barron:** Investigation, Writing – review & editing. **E.R. Gibney:** Writing – review & editing. **M. F. Ryan:** Investigation, Writing – review & editing. **M.J. Gibney:** Funding acquisition, Writing – review & editing. **J.W. Newman:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Dr. A.M. O'Sullivan:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

There are no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2021.102338.

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