Ontogeny of circulating lipid metabolism in pregnancy and early childhood: a longitudinal population study

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38 **Abstract (387 words)**

39 **Background**

40 There is mounting evidence that *in utero* and early life exposures may predispose an
41 individual to metabolic disorders in later life; and dysregulation of lipid metabolism is critical
42 in such outcomes. However, there is limited knowledge about lipid metabolism and factors
43 causing lipid dysregulation in early life that could result in adverse health outcomes in later
44 life. We studied the effect of antenatal factors such as gestational age, birth weight and mode
45 of birth on lipid metabolism at birth; changes in the circulating lipidome in the first four years
46 of life and the effect of breastfeeding in the first year of life. From this study, we aim to
47 generate a framework for deeper understanding into factors effecting lipid metabolism in
48 early life, to provide early interventions for those at risk of developing metabolic disorders
49 including cardiovascular diseases. .

50 **Methods and findings**

51 We performed comprehensive lipid profiling of 1074 mother-child dyads in the Barwon
52 Infant Study (BIS), a population based pre-birth cohort and measured 776 distinct lipid
53 species across 42 lipid classes using ultra high-performance liquid chromatography
54 (UHPLC). We measured lipids in 1032 maternal serum samples at 28 weeks' gestation, 893
55 cord serum samples at birth, 793, 735, and 511 plasma samples at six, twelve months, and
56 four years, respectively. The lipidome differed between mother and newborn and changed
57 markedly with increasing child's age. Cord serum was enriched with long chain poly-
58 unsaturated fatty acids (LC-PUFAs), and corresponding cholesteryl esters relative to the
59 maternal serum. Alkenylphosphatidylethanolamine species containing LC-PUFAs increased
60 with child's age, whereas the corresponding lysophospholipids and triglycerides decreased.
61 We performed regression analyses to investigate the associations of cord serum lipid species

with antenatal factors: gestational age, birth weight, mode of birth and duration of labor. Majority of the cord serum lipids were strongly associated with gestational age and birth weight, with most lipids showing opposing associations. Each mode of birth showed an independent association with cord serum lipids. Breastfeeding had a significant impact on the plasma lipidome in the first year of life, with upto 17-fold increases in a few species of alkyl diacylglycerols at 6 months of age.

Conclusions

This study sheds light on lipid metabolism in infancy and early childhood and provide a framework to define the relationship between lipid metabolism and health outcomes in early childhood.

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Introduction

The developmental origins of health and disease paradigm suggests that prenatal, perinatal, and postnatal heritable and environmental influences result in long-term developmental, physiological, and metabolic changes in major tissues and organs [1, 2]. These metabolic perturbations can contribute to later life disease risk, including cardiovascular diseases and related cardiometabolic conditions [3, 4]. Alterations to lipid metabolism are a key driver of metabolic disorders [5, 6]. The lipid profile in blood (the lipidome) provides an integrative measure of genetic and environmental exposures on circulating lipid metabolism and quantification of the lipidome offers a rapid and effective approach to identify biomarkers potentially predictive of several diseases [7]. Large population-based studies from our group and others have established specific lipids to be associated with cardiometabolic disorders including diabetes and cardiovascular diseases [8-11]. In contrast to adult data, little is known

about lipid metabolism in early life. Understanding the key determinants of early life lipid metabolism will inform the development of risk-stratification and early interventions.

The Barwon Infant Study (BIS) is a longitudinal Australian birth cohort with antenatal recruitment and repeated in-depth data and sample collection that is designed to facilitate a detailed mechanistic investigation of disease development within an epidemiological framework [12]. In this study, we applied a comprehensive lipidomic approach to understand the lipid metabolism during pregnancy and throughout the first four years of life. We utilized targeted UHPLC to measure 776 distinct individual lipid species across 42 distinct lipid classes. This aimed to increase understanding the lipid metabolism in newborns and infants in the first year of life, specially focusing on the association of cord serum lipids with antenatal factors: gestational age, birthweight, mode of birth and duration of labour. We also aimed to investigate the relationship between breastfeeding and plasma lipids at 6 months and 12 months of age. We provide a baseline characterisation of circulating lipids in the mother and newborn and changes in plasma lipids from birth to four years. In summary, the results from this study provide a framework for future studies to understand lipid metabolism in early life and provide early interventions for lipid dysregulation.

Methods

Research design and the cohort

The Barwon Infant Study is a pre-birth cohort (n = 1,074 mother-infant pairs, 72% Anglo-European) from the Barwon region of south-eastern Australia [12]. Women were recruited prior to 28 weeks of pregnancy. Infants born prior to 32 weeks, who developed a serious illness in the first week of life, or who had significant congenital or genetic abnormalities, were excluded. Participants were reviewed at birth and at 1, 6, 9, 12, 24 and 48 months. Maternal serum samples were collected at 28 weeks gestation. Child serum or plasma

samples were collected at birth, 6 months, 12 months and 4 years. Data on maternal age, birth order, prenatal weight, and antenatal comorbidities were collected from questionnaires and hospital records, and by standardized clinical examination. Cord blood was collected at birth in a serum clotting tube, samples were centrifuged within 2 h of collection and the serum was separated and stored at -80°C . Maternal pre-pregnancy BMI was calculated from self-reported pre-pregnancy weight and directly measured maternal height at the first study visit (28–32-week gestation). Birth anthropometric measures (birthweight, length, and head circumference) were collected within the first 2 days of life. Anthropometric measurements were also obtained at birth, 6 months, 12 months and 4 years. BMI at each time point was calculated by weight (kilograms) / height (meters)². Ethics approval for this study was granted by the Barwon Health Human Research and Ethics Committee (HREC 10/24).

Lipidomic profiling

Lipid extraction: Lipid extraction was performed as described previously in Alshehry *et al.* [13]. In brief, 10 μL of plasma was mixed with 100 μL of butanol: methanol (1:1) with 10mM ammonium formate containing a mixture of internal standards. Samples were vortexed, sonicated for an hour and then centrifuged (14,000 $\times g$, 10 min, and 20°C) before transferring the supernatant into sample vials with glass inserts for analysis.

Liquid chromatography- tandem mass spectrometry: Lipidomics was performed as described previously in [9] with adaptations for a dual column set up. Analysis of plasma extracts was performed on an Agilent 6490 QQQ mass spectrometer with an Agilent 1290 series HPLC system and two ZORBAX eclipse plus C18 column (2.1 $\times$ 100mm 1.8mm, Agilent) with the thermostat set at 45°C . Mass spectrometry analysis was performed in both positive and negative ion mode with dynamic scheduled multiple reaction monitoring (MRM). A detailed list of MRMs and internal standards is provided in Supplementary File 1A.

The running solvent consisted of solvent A: 50% H₂O / 30% acetonitrile / 20% isopropanol (v/v/v) containing 10mM ammonium formate and solvent B: 1% H₂O / 9% acetonitrile / 90% isopropanol (v/v/v) containing 10mM ammonium formate. To avoid peak tailing for acidic phospholipids, we passivized the instrument prior to each batch by running 0.5% phosphoric acid in 90% acetonitrile for 2 hours and subsequently flushing the system with 85 % H₂O / 15 % acetonitrile prior to sample run.

We utilized a stepped linear gradient with a 12.9 minutes cycle time per sample and a 1 µL sample injection. To increase throughput, we used a dual column set up to equilibrate the second column while the first is running a sample. The sample analytical gradient was as follows: starting with a flow rate of 0.4 mL/ minute at 15% B and increasing to 50% B over 2.5 minutes, then to 57% over 0.1 minutes, to 70% over 6.4 minutes, to 93% over 0.1 minute, to 96% over 1.9 minutes and finally to 100% over 0.1 minute. The solvent was then held at 100% B for 0.9 minutes (total 12.0 minutes). Equilibration was started as follows: solvent was decreased from 100% B to 15% B over 0.2 minutes and held for an additional 0.8 minutes for a total run time of 12.9 minutes per sample. The next sample is injected, and the columns are switched.

The following mass spectrometer conditions were used: gas temperature, 150°C, gas flow rate 17 L/ min, nebulizer 20 psi, sheath gas temperature 200 °C, capillary voltage 3500 V and sheath gas flow 10 L/ min. Isolation widths for Q1 and Q3 were set to “unit” resolution (0.7 amu).

Plasma QC (PQC) samples consisting of a pooled set of plasma samples taken from 6 healthy individuals and extracted alongside the study samples were incorporated into the analysis at 1 PQC per 20 plasma samples. Technical QC samples (TQC) consisted of PQC extracts which were pooled, then split into individual vials to provide a measure of technical variation from

the mass spectrometer only. These were included at a ratio of 1 TQC per 20 plasma samples. TQCs were monitored for changes in peak area, width, and retention time to determine the performance of the LC-MS/ MS analysis and were subsequently used to account for differential responses across the analytical batches. To align the results to any future datasets, the NIST 1950 SRM sample (Sigma) was included as a reference plasma sample, at a rate of 1 per 40 samples.

Relative quantification of lipid species was determined by comparison to the relevant internal standard. Lipid class total concentrations were calculated as the sum of individual lipid species concentrations, except in the case of triacylglycerol (TG) and alkyldiacylglycerol (TG(O)) species, where we measured both neutral loss [NL] and single ion monitoring [SIM] peaks, and subsequently used the abundant but less structurally resolved [SIM] species concentrations for summation purposes when examining lipid class totals. Any lipid differences based on the matrix must be kept in mind as serum samples were used during gestation, and at birth vs plasma samples at other time points. Several studies have reported matrix-associated differences in species of lysophospholipids, diacylglycerols, free long chain fatty acids, and oxidised fatty acids [14, 15]. However, these differences are unlikely to reflect biological processes in the body [16]. These differences could be reflected in change in lipid levels observed at birth and 6 months, but not affect the association with outcomes.

Data processing

Lipidomic analysis was performed across 11 batches of ~500 samples with quality control samples analysed every 20 samples. The chromatographic peaks were integrated using the Mass Hunter (B.07.00, Agilent Technologies) software and assigned to specific lipid species based on MRM (precursor/product) ion pairs and retention time. Upon completion of quantification, lipidome data for 777 lipid features in 4007 samples was available across 11

batches. Zero values (values beneath the mass spectrometer's detection threshold) were replaced by $1/10^{\text{th}}$ the minimum value for the concerned lipids in the corresponding sample types. We then aligned the lipid concentrations across different batches by median centring the PQC quality control samples, and additionally scaled all batch-wise standard deviations for biological samples (on the log scale) to the mean standard deviation. We visually assessed lipids for large-scale technical variations (such as groups of outlier measurements, effects of drift or lipid hydrolysis over batch run duration), and re-quantified affected lipids where possible, and excluding them or selectively setting some values to missing where not. Missing values were then imputed using a k-nearest neighbour approach in the samples space. We used two different approaches for outlier sample detection. On one hand, lipid concentrations were log-transformed, and Z-scored, and absolute Z-scores were summed for each sample as a measure of their extremeness. Samples showing sums above the 95th percentile were flagged as potential outliers. On the other hand, the Z-scores were used in a Principal Components Analysis (PCA), and the 7 first principal components were retained as explaining the most variance. In the space defined by these components, each sample's distance to the origin was calculated as a measure of the sample's extremeness. Samples with a distance greater than the 95%-ile were flagged as potential outliers. Samples flagged by both approaches were declared outliers and removed from further analysis. Further inspection revealed that 16 samples were "missed injections" on the mass spectrometer, 27 cord serum samples were contaminated by maternal blood, these which were excluded, leaving 3964 samples in total. Ultimately, we retained 776 lipid measures across all batches. SIM-based lipid measures were not included in lipid species-level analyses, leaving 733 species.

Statistical analysis

208 All statistical analyses were carried out in R (3.5.0 or 4.0.3). Plasma and cord serum lipid
209 concentrations were log10 transformed prior to statistical analysis. PCA was performed using
210 package FactoMineR using default settings.

211 Associations between maternal/ infant/ children characteristics and lipid species were
212 determined using multiple linear regression, adjusting for appropriate covariates in each
213 analysis [17]. All the analyses at birth using cord serum were adjusted for birth weight,
214 gestational age, mode of birth, duration of labor, sex, maternal pre-pregnancy BMI, maternal
215 gestational diabetes mellitus (GDM), maternal education, maternal age, birth order and
216 lipidomics run batch.

217 Longitudinal analyses (associations of lipid species with breastfeeding, sex, and weight
218 across infancy and early childhood) were performed using linear regression models of
219 breastfeeding/ sex/ weight against the log-transformed abundance of lipid species at each
220 time point, adjusted for sex and age. Breastfeeding status (y/n) at 6 months and 12 months
221 were taken from questionnaires at these time points. We observed that gestational age
222 influences lipids at 6 months and not at 12 months and hence adjusted for gestational age at
223 the 6-month time point. The effective sample size varied slightly with each time point and
224 between models due to missing samples or missing values for some covariates. Beta
225 coefficients and 95% confidence intervals were then converted to percentage difference
226 (percentage change = $(10^{\beta\text{-coefficient}} - 1) \times 100$) to facilitate interpretation of results. All
227 p-values were corrected for multiple comparisons, per model term, using the method of
228 Benjamini and Hochberg [18]. Differences between the maternal antenatal plasma lipidome
229 and the cord serum lipidome were tested using paired Wilcoxon Ranked Sum tests.

230 *Data Representation:* The results from the association analyses are presented as forest plots
231 in Figures 2-4 and 6 where each lipid species (or class) is represented by a marker showing

either a positive association (increases with an increase in the outcome) or a negative association (decreases with an increase in the outcome). The colour of the markers relate to the significance of the association as indicated in the figure legends. The lipid species are grouped into lipid class and subclass and ordered into categories (sphingolipids, phospholipids and other lipids) each separated by a grey dotted line.

Results (2266)

The baseline characteristics of the complete cohort are shown in Supplementary File 1B. In this study, we utilized serum samples of the mothers at 28 weeks' gestation, cord serum samples at birth, and plasma samples at six, twelve months, and four years.

Serum/Plasma lipidomics: We measured the plasma lipid species in a total of 3964 samples: 1032 in mothers, 893 at birth, 793 at six months, 735 at twelve months, 511 at four years (Fig 1a). Of the measured lipid species and classes, we retained 733 lipid species across 37 classes for further analyses. The median coefficient of variation (CV) for the lipid measures based on the plasma quality control (PQC) samples was 10.1%, with 92.6% of lipid measures showing a CV <20%.

The serum/plasma lipidome differs between mothers, newborns, and infants. Overall lipid levels increased with age: maternal serum had higher total lipid levels than infant plasma, which in turn had higher levels than cord serum (Fig 1b). Principal Component Analysis (PCA) of the lipidomic data from all participants at all the time points revealed a clear separation of maternal, newborn, and infant samples across the first and second principal components (Fig 1c). A PCA of the infant samples showed further separation of the 6, 12- and 4-year time points (Fig 1d, 1e).

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Fig. 1 Snapshot of the Barwon Infant Study lipidomics. **A**, The BIS timeline and final lipidomic sample numbers at each time point. **B**, Total lipid concentration in maternal and infant sample groups. **c**, PCA of the lipidomic measures at all the time points, marker size is proportional to the median log total lipid level (%) **d**, PCA (PC1 vs PC2) of the lipidomic measures at 6 months, 12 months and 4 years, marker size as previously stated. **E**, PCA (PC2 vs PC3) of the lipidomic measures at 6 months, 12 months and 4 years, marker size as previously stated. For all panels: Maternal serum, M28, pink colour; newborn cord serum, birth, purple colour; 6-month plasma, blue colour; infant 12-month plasma, orange colour; 4-year plasma, green colour.

272 **Gestational age and birthweight influence newborn cord serum lipids.** 461 (of 733, 63%)
 273 cord lipid species were associated with gestational age and 299 (of 733, 41%) lipid species
 274 were associated with birth weight (Fig 2a, 2b). Figure 2 – figure supplement 1 explains lipid
 275 metabolism pathways. Gestational age was modestly correlated with birth weight ($r = 0.45$).
 276 However, the lipid profile associated with birth weight was discordant with the lipid profile
 277 associated with gestational age as indicated by the plot of the beta coefficients for gestational
 278 age against the beta coefficients for birth weight ($y = -10.535x + 0.0101$, $r^2 = 0.714$, Figure 2
 279 – figure supplement 2). 591 (of 733, 80%) lipid species had an opposing association with
 280 gestational and birth weight. Species of di- and tri-acylglycerol, alkyldiacylglycerol,
 281 acylcarnitine and free fatty acids (FFA) increased with gestational age but decreased with
 282 birth weight. Phospholipid species containing either an odd-numbered straight, or a methyl
 283 branched fatty acid (15-methylhexadecanoic acid, 15-MHDA) were positively associated
 284 with gestational age (Figure 2 – figure supplement 3a) but negatively associated with birth
 285 weight (Figure 2 – figure supplement 3b). While lysophospholipid species as a class were
 286 negatively associated with gestational age, we observed elevated levels of odd and branched
 287 chain fatty acid containing lysophosphatidylcholine species: LPC (19:0) [sn1] and [sn2], LPC
 288 (15-MHDA) [sn1] and [sn2] with gestational age that had an opposite association with birth
 289 weight. Cholesteryl esters (CE) also showed a similar trend as seen by elevated levels of CE
 290 (15:0) and CE (17:0). Alkyl and alkenylphosphatidylethanolamine species increased with
 291 both gestational age and birth weight. Species of lysophospholipids, alkyl and alkenyl
 292 phosphatidylcholine, dihydroceramide, hexosylceramide, cholesteryl ester and
 293 dehydrocholesteryl ester decreased with gestational age and increased with birth weight.
 294 Complete associations between gestational age, birth weight and gender with lipid species
 295 and lipid classes at birth are detailed in Supplementary File 1C and 1D.

Fig 2| The newborn cord serum lipidome is influenced by gestational age (GA) and birth weight (BW). **A**, Estimated percentage difference of the lipid species per day increase in gestational age, determined using linear regression, adjusted for weight, sex, mode of birth, duration of labor, maternal pre-pregnancy BMI, GDM, maternal education, birth order and lipidomics run batch. **B**, Estimated percentage difference of the individual lipid species per kilogram increase in birthweight, determined using the same model as **a**.

Each circle on the plot represents a lipid species, grey open circles show non-significant lipid species ($p > 0.05$), white closed circles show significant lipid species ($p < 0.05$), the top 10 lipid species (10 lowest p values) are shown in blue circles. Each red diamond represents a lipid class. All p -values were corrected for multiple comparisons. The horizontal bars (error bars) are shown for the significant lipid species (white closed circles), the top ten lipid species (blue circles), corrected p -value $< 2.88 \times 10^{-24}$ and 1.81×10^{-15} respectively and the lipid classes (red diamonds). The error bars represent 95% confidence intervals. Grey dotted lines separate sphingolipids, phospholipids and other lipids.

Mode of birth and duration of labor are associated with specific signatures in the cord

serum lipidome. In this study, we compared four different birth modes: assisted vaginal birth (assisted VB, n = 214) (including both forceps- and vacuum-assisted birth), scheduled 15aesarean (CS, n = 178) and unscheduled 15aesarean (unscheduled CS, n = 155), with unassisted vaginal birth (VB, n = 525) as reference. Unscheduled CS accounted for all the emergency CS including breech births, while individual data on the reason for the emergency was not recorded. 214 lipid species (of 733, 29%) showed evidence of association with at least one mode of birth (Fig. 3). Lipid species of acylcarnitine, di- and triacylglycerols were elevated in both assisted VB and unscheduled CS relative to VB, while lipid species of free fatty acids, lysophosphatidylcholine decreased in both CS and unscheduled CS birth modes compared to VB (Fig. 3a, b, c, and Supplementary File 1E and 1F). Unscheduled CS birth had an intermediate profile where 74% of the lipid species showed a similar association to that observed with assisted VB ($r^2 = 0.543$, Figure 3 – figure supplement 1), while 57% of the lipid species showed the same trend as observed with the scheduled CS birth mode.

36 lipid species (of 733, 5%) showed evidence of association with duration of labor independent of the mode of birth. Similar to assisted VB and unscheduled CS, we observed elevated levels of acylcarnitine, di and triacylglycerols with longer duration of labor.

328 We then investigated the association of maternal lipids at 28 weeks gestation with mode of

329 birth and duration of labour. However, none of the maternal lipids were significantly
330 associated with any of the delivery modes or the duration of labour (Supplementary File 1G
331 and 1H).

332 **Fig 3| Mode of birth influences the newborn cord serum lipidome.** Linear regression of
333 the lipidome on delivery mode, each mode: assisted vaginal birth (assisted VB), scheduled

cesarean (CS), unscheduled cesarean (unscheduled CS) being compared to unassisted vaginal birth (VB), adjusting for birth weight, gestational age, sex, duration of labor, maternal pre-pregnancy BMI, GDM, maternal education, birth order and lipidomics run batch. Results are shown as % difference in lipids between each mode and VB. **a**, Lipid species associated with assisted vaginal delivery. **b**, Lipid species associated with scheduled cesarean delivery. **c**, Lipid species associated with unscheduled cesarean delivery. **d**, Lipid species associated with duration of labor, independent of the mode of delivery. Each circle on the plot represents a lipid species, grey open circles show non-significant lipid species ($p > 0.05$), white closed circles show significant lipid species ($p < 0.05$), the top 10 lipid species (10 lowest p values) are shown in blue circles. Each red diamond represents a lipid class. All p-values were corrected for multiple comparisons. The horizontal bars (error bars) are shown for the significant lipid species (white closed circles), the top ten lipid species (blue circles), Corrected p value $< 1.64 \times 10^{-03}$, 2.16×10^{-12} , 2.27×10^{-03} , 1.8×10^{-02} , respectively and the lipid classes (red diamonds). The error bars represent 95% confidence intervals. Grey dotted lines separate sphingolipids, phospholipids and other lipids.

Long chain poly unsaturated fatty acids (LC-PUFAs) are enriched in newborn cord serum. We performed paired Wilcoxon tests to investigate the relationship between maternal and newborn lipidome. While 80% of the lipid species were significantly higher in the maternal serum, the lipid species of sphingosine and sphingosine-1-phosphate, phosphatidylserine, acylcarnitine, dehydrocholesteryl ester, glycosphingolipids and FFAs were higher in the newborn lipidome (Fig 4a). Omega-3 and omega-6 LC-PUFAs: arachidonic acid (20:4, n-6), eicosapentanoic acid (20:5, n-3), adrenic acid (22:4, n-6), docosapentaenoic acid (DPA, 22:5, n-6), docosahexaenoic acid (DHA, 22:6, n-3) were enriched in the cord serum (Fig. 5b, 5c). While lipid classes such as cholesteryl ester, lysophosphatidylcholine and triglycerides were higher in the maternal plasma individual lipid species containing LC-PUFAs were higher in cord serum. (Fig 4b, 4c, Supplementary File 1I and 1J).

Fig. 4| Differences in maternal and cord serum lipid species. Paired Wilcoxon tests were performed, and results are shown as fold differences of mothers relative to infants. **a**, Fold differences in lipid concentrations between maternal and cord serum. Cholesteryl esters and FFA classes are highlighted in boxes. **b**, Selective enrichment of cholesteryl esters containing long chain polyunsaturated fatty acids in cord serum. **c**, Selective enrichment of long chain polyunsaturated fatty acids in cord serum. Each circle on the plot represents a lipid species, grey open circles show non-significant lipid species ($p > 0.05$), white closed circles show significant lipid species ($p < 0.05$), the top 10 lipid species (10 lowest p values) are shown in blue circles. Each red diamond represents a lipid class. All p-values were corrected for multiple comparisons. The horizontal bars (error bars) are shown for the significant lipid

species (white closed circles), the top ten lipid species (blue circles), corrected p value $< 5.06 \times 10^{-261}$, and the lipid classes (red diamonds). The error bars represent 95% confidence intervals. Grey dotted lines separate sphingolipids, phospholipids and other lipids.

The circulating lipidome changes over the first four years. We performed paired t-tests to study the changes in lipid concentration at different time points (Fig 5). 75% 549 (of 733, 75%) of the lipid species were higher at four years than at birth, with LC-PUFA containing alkenyl phosphatidylethanolamine species having the greatest increase. 123 lipid species (of 733, 17 %) consistently increased at all the time points from birth to four years, whereas 68 lipid species decreased across all the time points. There was a consistent decrease in LC-PUFA containing lysophospholipids and an increase in odd and branched chain fatty acids, and phospholipids containing these fatty acids, across all the time points.

Birth to six months: Majority of lipid species (558 of 733, 76%) were higher at six months compared to at birth. We observed the greatest increase in species of alkenylphosphatidylethanolamine and alkyldiacylglycerol and the largest decrease in 20:4 and 20:6 containing lysophosphatidylcholine, lysophosphatidylethanolamine and cholesteryl esters (Supplementary Files 1K and 1L).

Six to twelve months: 363 (of 733, 50 %) lipid species increased in concentration from six to twelve months. Odd and branched chain phospholipids showed the greatest increase while species of alkyldiacylglycerol and cholesteryl esters showed the greatest decrease (Supplementary Files 1K and 1L).

272 (of 733, 37%) lipid species consistently increased from birth to 6 months and from 6 to 12 months, while 90 (of 733, 12%) lipid species showed a consistent decrease. 365 (of 733, 50%) lipid species had an opposing trend at these time points. Lipid species of dihydroceramide, phospholipids, glycopospholipids, lysophospholipids, FFA, alkyl and alkenylphospholipids, di- and tri-acylglycerols, acylcarnitine, sphingomyelin, mono and tri-

398 hexosyl ceramides, ceramide-1-phosphate increased from birth to six months but decreased
399 from six to twelve months. Lipid species of sphingosine decreased from birth to six months
400 but increased from six to twelve months.

401 *Twelve months to four years:* 284 (of 733, 37%) of the lipid species were higher at four years
402 than at 12 months. We saw the highest increase in cholesteryl ester CE 20:5 and largest
403 decrease in 22:6 containing di and triacylglycerols (Supplementary Files 1K and 1L).

404 Some lipid species showed variable changes as the child aged. Lipid species of
405 dehydrocholesterol, alkenyllysophosphatidylcholine that decreased from birth to 6 months,
406 and from 6 to 12 months, increased at four years. Lipid species of ceramide,
407 phosphatidylethanolamine and phosphatidylglycerol that increased from birth to 6 months
408 and 6 to 12 months, decreased at four years. Lipid species of sphingosine decreased from
409 birth to 6 months, increased at 12 months, and decreased again at four years (Figure 5 –
410 figure supplement 1).

411 Overall omega-3 and omega-6 LC-PUFAs: 20:3, 20:4, 22:4, 22:5 and 22:6 that were higher at
412 birth, decreased by the time the infant reached six months, and continued decreasing further
413 until four years. Most of the omega-3 PUFA cholesteryl esters: CE (22:5) (n-3) and 22:6
414 decreased in the first year but increased at 4 years except for CE (20:5) that decreased at 6
415 months but increased at 12 months and 4 years. Omega-6 PUFA cholesteryl esters: CE
416 (20:4), CE (22:4) and CE (22:5) (n-6) decreased at 6 months but increased at 12 months and 4
417 years (Supplementary File 1M).

418 Additionally, we performed time-series clustering to capture the changes of all the lipid
419 species across all the time points. We utilised Dynamic Time Warping (DTW) distance as a
420 dissimilarity measurement [19]. We used the partitional clustering method with partitions
421 around medoids (PAM) to cluster the lipid species. We identified 10 different lipid clusters,

each of them showing unique lipid trajectories over the first four years of life (Figure 5 – figure supplement 2, Supplementary File 1K).

Cluster one, consisted of 63 lipid species, comprising of ceramide species and LC-PUFA containing phospholipid species, all of which increased from birth to six months, and then stabilised by the time the child reached twelve months. Cluster two consisted of 149 lipid species of which phospholipids such as the odd chain phosphatidylcholine PC(31:0) and branched chain and LC-PUFA containing phosphatidylethanolamine species: PE(15-MHDA_20:4) and PE(15-MHDA_22:6) decreased at six months, increased at twelve months and decreased at four years. These PE species were also positively associated with birth weight in cord serum. Lysophospholipids containing 18:1 and 18:2 fatty acids, increased at six months, decreased at twelve months and increased as the child reached four years. In adults, these lysophospholipids are generally associated with higher BMI. LC-PUFA containing phospholipids, especially alkenylphosphatidylethanolamine and alkylphosphatidylcholine increased at six months, but decreased at twelve months and four years. Similarly, alkyldiacylglycerols increased at six months and decreased thereafter. Cluster three comprised of 143 lipid species. Species of phospholipids in cluster 3 increased at all time points while ceramide and phospholipid species containing odd chain fatty acids increased until twelve months and decreased by the time the child reached four years. Cluster four consisted of 41 lipid species. The pattern was similar to cluster two, with species of LC-PUFA containing phosphatidylethanolamine species increasing from birth to six months and decreasing at twelve months and four years. Cluster five consists of 60 lipid species. Most of the lipid species in cluster five increased until the child reached six months but decreased as the child reached twelve months and further. Cluster six consisted of 38 lipid species, mostly exhibiting mixed patterns. Cluster seven consisted of 51 lipid species. As seen in cluster 2, the alkyldiacyl glycerols increased at 6 months and decreased at 12 months and 4 years, but

the alkenyl phosphatidylethanolamine species increased until 12 months and decreased at four years. Cluster eight consisted of 33 lipid species, with species of lysophospholipids, cholesterylesters, and fatty acids showing a consistent decrease across all timepoints. Cluster nine consisted of 98 lipid species and showed a similar profile to Cluster 1. Ceramide and sphingomyelin species containing 23:0 and 24:0 long chain fatty acids increased across all the time points. Additionally, alkyl and alkenylphosphatidylethanolamine species containing LC-PUFAs (18:2, 20:3 and 20:4) increased across all the time points. Cluster ten consisted of 57 lipid species. Fatty acids 20:0 and 22:0 containing hexosylceramides and lysophosphatidylcholines followed a peculiar pattern of increasing until 6 months decreasing at 12 months, and later increasing as the child reaches 4 years. Acylcarnitine and fatty acid species, that were elevated in cord serum, compared to maternal serum, increased until 6 months and later decreased at 12 months and further at 4 years suggesting a change in energy metabolism over the first 4 years.

Fig. 5| Lipidomic profile changes in the first four years. Paired t-tests were performed in infants with repeat measures (birth - 6 months n=646, 6 - 12 months n=628, 12 months – 4 years n=418), and results are shown as log2 fold change (FC). The outermost circle represents the changes in lipid concentration from birth to six months (axis range: -4 to 6), middle circle represents changes in lipid concentration six months to 12 months (axis range: -2 to 2) and the inner most circle represents the changes in lipid concentration from 12 months to four years (axis range: -1 to 1).

Breastfeeding impacts lipid metabolism in the first year of life.

We investigated the association of breastfeeding with plasma lipids at 6 months and 12 months. Of the 776 lipid species 664 lipid species (90%) were significantly associated with breastfeeding at 6 months, and 438 (of 773, 65%) were significantly associated at 12 months (Supplementary Files 1N and 1O). Of particular note, species of alkyl and alkenylphospholipids (plasmalogens) and alkyldiacylglycerides (TG-O) were markedly elevated in breastfed infants. At a class level these elevations were of the order of 2-4 fold, while individual species of alkyldiacylglycerols: TG(O-54:2) [NL-17:1], TG(O-54:2) [NL-18:1], TG(O-52:1) [NL-18:1] were elevated 17 – 19 fold at 6 months. However, at 12 months, this effect size was only 2-4 fold, given the introduction of a wide variety of foods along with breast milk (Fig 6). In addition to the dramatic increase in these ether lipid species, we also observed an increase in many species containing odd- and branched-chain fatty acids such as phosphatidylcholine PC(33:0), sphingomyelin SM(d19:1/24:1) and others. These odd- and branched-chain fatty acids represent a novel, potentially bioactive, class of lipids enriched in breast fed infants.

Fig 6| Breastfeeding influences the infant plasma lipidome. Linear regression of the lipidome on breastfeeding status at 6 months and 12 months adjusting for age, sex, birth weight, gestational age (at 6 months), maternal pre-pregnancy BMI, GDM, maternal education, birth order. Results are shown as fold difference in lipids between breastfed and non-breastfed infants. **a**, Lipid species associated with breastfeeding at 6 months. **b**, Lipid species associated with breastfeeding at 12 months. Each circle on the plot represents a lipid species, grey open circles show non-significant lipid species ($p > 0.05$), white closed circles show significant lipid species ($p < 0.05$), the top 10 lipid species (10 lowest p values) are shown in blue circles. Each red diamond represents a lipid class. All p-values were corrected for multiple comparisons. The horizontal bars (error bars) are shown for the significant lipid species (white closed circles), the top ten lipid species (blue circles), Corrected p value $< 5.96 \times 10^{-189}$, 1.04×10^{-38} respectively. The error bars represent 95% confidence intervals.

Sex-specific changes in the circulating lipidome over the first four years. We investigated the influence of sex on lipid metabolism in early childhood. We found 218 lipid species (of 733, 29%) to be significantly associated at birth, 214 (of 733, 29%) at 6 months, 62 (of 733, 8%) at 12 months and 82 (of 733, 11%) at 4 years. We found significant differences in lipid species of ceramide, sphingomyelin, acylcarnitine, lysophosphatidylcholine, lysophosphatidylethanolamine, alkyllysophosphatidylcholine and triglycerides between boys and girls (Supplementary Files 1P and 1Q).

Discussion (1446 words)

This is the first and largest study of the lipidome from birth and early childhood in a population-derived cohort. The lipidome was different between mother and child and changed markedly with child's age. We identified that gestational age and birth weight were associated with cord serum lipids. Most of the lipid associations with gestational age were in the opposing direction to the associations with birth weight. Mode of birth was also associated with the cord serum lipidome, and the profile differed from that observed for gestational age and birthweight. There were marked changes in the ontogeny of the plasma lipidome from birth to four years of age: LC-PUFA, and cholesteryl esters containing LC-PUFAs were enriched in cord serum relative to the maternal serum. LC-PUFA containing lipid species altered between birth and four years with an enrichment in alkenylphosphatidylethanolamine species and a reduction in lysophospholipids and triglycerides. Concentrations of free LC-PUFAs decreased at four years, but the corresponding cholesteryl esters increased. Alkenylphosphatidylethanolamine species and alkyl diacylglycerol species were significantly elevated in breastfed infants at 6 months and 12 months.

522 There has been an 30% increase in the incidence of late preterm births (LPIs) since 1980s.
523 which now account for 75% of all pre-term births. There is increasing epidemiological
524 evidence suggesting infants born late pre-term (34 – 36 weeks) and early term (37 – 38
525 weeks) are at higher risk of a host of adverse health outcomes including respiratory disorders,
526 increased and prolonged hospitalizations, and developmental delay [20-22]. On the other
527 hand, the studies on association of gestational age with future health risk have been
528 inconclusive. For example, Power *et al.*, examined the associations between gestational age
529 and blood pressure, HbA1c, clinical lipids, and BMI at 44 - 45 years of age in the 1958
530 British birth cohort. They identified that gestational age was inversely associated with blood
531 pressure but there was no clear evidence of an association between gestational age and BMI
532 or total cholesterol among men or between gestational age and HbA1c or LDL-cholesterol,
533 HDL-cholesterol and triglycerides in either sex [23].

534 In our study, we found di- and tri-acylglycerols were positively associated with gestational
535 age, which reflect an evolutionary adaptation in preparation for the metabolic stress of labor,
536 a period of high energy expenditure [24] [25]. A concomitant increase in branched chain fatty
537 acids and corresponding phospholipids, a downstream product of the mitochondrial
538 catabolism of branched chain amino acids along with elevated levels of acylcarnitines, both
539 suggest increased mitochondrial activity in babies with greater gestational age [26].

540 Gestational age and birth weight showed overall inverse lipidomic profiles that were largely
541 independent of each other. In infants with higher birth weight, the observed lower level of
542 triglyceride levels could reflect better packaging within the adipose tissue vs free availability
543 in the circulation for energy mobilization.

544 The decrease in cholesteryl esters and dehydrocholesterols suggest a decrease in cholesterol
545 synthesis with increasing gestational age. This may relate to increased use of free fatty acids

for energy production and so less for cholesteryl ester production but might also relate to a downregulation of the cholesterol biosynthesis pathway as the dehydrocholesterol is a precursor to cholesterol.

There were significant associations with multiple sphingolipid species, but overall these were very small, showing only 2-3 % difference for a 10-day difference in gestational age. Thus while these lipids are known to associate with cardiometabolic outcomes in adults, the differences at birth cannot yet be ascribed to specific outcomes or risk. Further analyses will be required to define these relationships.

The lipid profile associated with gestational age resembles the lipid profile associated with BMI in adults. For example, in adults, acylcarnitines, di and tri-acylglycerols are positively associated with BMI and body weight [27] like that seen in babies with higher gestational age. One possible explanation is that these lipid classes are involved in energy mobilisation and hence more abundant in circulation thereby acting as a source of free fatty acids which are required for various biological pathways [28] [29]. Understanding the changes in lipid metabolism during the final stages of gestation may provide mechanistic insights into the increasingly recognised long-term health sequelae of preterm and late preterm births [30].

These results have also been observed in an ethnically diverse cohort, Growing Up in Singapore Towards healthy Outcomes (GUSTO), revealing that these lipidome changes associated with birthweight may be largely independent of race/ ethnicity [31].

There are robust associations between mode of birth and child health outcomes, including risk of future overweight and obesity [32, 33]. Elevated levels of di- and tri-acylglycerols and acylcarnitine in assisted VB, unscheduled CS and prolonged labor indicate that stress *in utero* and during birth increases energy mobilization for high energy expenditure in these modes of birth [34-36]. However, a decrease in circulating free fatty acids and triacylglycerols in

570 scheduled CS may also be a result of mothers often being prohibited from consuming food
571 and drink before the procedure leading to a fasting state and so decreased availability of free
572 fatty acids for transfer across the placenta. Additionally, mode of birth also affects the
573 neonate's gut microbiota [37]. Newborns delivered vaginally show a higher diversity of
574 bacteria than those born by CS [37, 38]. The specific lipidome signature associated with each
575 mode of birth may reflect the differences in the gut microbiome, although whether this could
576 manifest at the time of delivery is uncertain. Considering data suggesting an increased risk of
577 metabolic disorders later in life associated with mode of birth [33, 39], it is important to
578 understand differences in lipid metabolism by birth mode.

579 *Lipid differences in mother and newborn cord serum:* Compared to the mother, we observed
580 elevated levels of LC-PUFAs in cord serum which are critical for *in utero* brain development
581 as well as the rapid brain development in the first year of life [40, 41]. The elevated levels of
582 di- and tri-acylglycerols, and phospholipids observed in the maternal circulation most likely
583 act as a source of fatty acids for the foetus. Lipoprotein lipase and endothelial lipase in the
584 syncytiotrophoblast hydrolyse circulating maternal triglycerides and phospholipids
585 respectively to provide the pool of fatty acids for the fetus [42]. Free fatty acids in the
586 syncytiotrophoblast basal membrane are transferred to foetal circulation directly through
587 facilitated diffusion or using fatty acid carriers (FAT/CD36) and fatty acid binding proteins
588 (FABPpm, FATP4). In the foetal circulation, these fatty acids then bind to a fetoprotein and
589 are transported to the liver, where they are re-esterified into complex lipids and transported
590 into circulation [43, 44].

591 LC-PUFA containing phosphatidylcholine species were also elevated in maternal serum.
592 These phospholipids undergo a *sn*-1 cleavage by endothelial lipases in the
593 syncytiotrophoblast producing LC-PUFA lysophosphatidylcholine species which are then

transported across the placenta by the major facilitator superfamily domain containing 2A (MFSD2a) transporter proteins explaining the elevated levels of LC-PUFA containing LPCs in cord serum [45]. Elevated levels of LC-PUFA containing cholesteryl esters in the cord serum, might also be acting as an additional source of LC-PUFAs for the foetus.

Recent studies have shown altered placental function and thereby altered lipid transfer in mothers with gestational diabetes and higher pre-pregnancy BMI [46, 47]. However, long term ramifications of maternal metabolic conditions on offspring health are currently unknown. Our study contributes to the understanding of trans-placental transfer of specific lipid species. Further studies on how maternal metabolic conditions may impact this transfer may provide insight into perturbations in lipidome *in utero* and its long-term health consequences.

Changes in lipid metabolism in the first four years of life: We observed pronounced changes in the lipid profiles in infancy and early childhood. Elevated levels of LC-PUFAs, cholesteryl esters and lysophospholipids seen in the cord serum started to diminish as the child reached six months of age. However, at 12 months and four years of age, we observed an increase in odd chain, essential fatty acids and LC-PUFA containing phospholipids, which could be acting as a source of PUFA. Recent studies have shown LC-PUFA deficiency in several childhood disorders such as asthma, cystic fibrosis, attention-deficit/hyperactivity disorders (ADHD), obesity and diabetes [40, 41]. A balanced intake of omega-6 and omega-3 fatty acids is essential for homeostasis and normal development throughout the life cycle. A diet rich in omega-6 LC-PUFAs can shift the metabolism to an atherogenic/ diabetic state [48]. On the other hand, the beneficial effects of omega-3 LC-PUFAs also depend on complex interaction between different nutrients, and polymorphism in genes involved in omega-3 fatty acid metabolism.

618 Clustering the lipids across all the time points revealed clear signatures of breastfeeding in
619 cluster 2, 7 and 9 with lipid species of alkenylphosphatidylethanolamine and
620 alkyldiacylglycerols showed remarkable increase at 6 months. Since the lipid clusters are
621 designed as change in lipids over time, it is not possible to find how they are correlated with a
622 particular outcome at one time point, rather we plan to investigate the association of these
623 lipid clusters with growth trajectories, thereby enabling us to capture the lipid trajectories
624 over time (manuscript in preparation).

625 Understanding changes in the lipidome early on in life provides a window of opportunity for
626 early intervention and decrease the risk of future metabolic disorders.

627 It is well established that breast milk is rich in polyunsaturated fatty acids (PUFA). LC-
628 PUFAs, especially arachidonic acid (20:4, n-6), eicosapentaenoic acid (20:5, n-3), and
629 docosahexaenoic acid (22:6, n-3) acid, have been shown to be critical for brain development
630 and function in early life. While the actual mechanisms of how breastfeeding is associated
631 with neurodevelopment remain unknown, LC-PUFAs in breast milk have been postulated to
632 account for the effect of breastfeeding and improved cognition. We observed elevated levels
633 of multiple lipid species containing these LC-PUFA in breastfed infants at both 6 months and
634 12 months. In addition, we observed a marked enrichment of alkenyl
635 phosphatidylethanolamine and alkyldiacylglycerol species in breast fed, relative to formula
636 fed, infants. Dysregulation of lipid metabolism is recognized as a primary driver of obesity
637 and more recently of inflammation and immune regulation. Lipids make up 3% of the total
638 breast milk composition. A small but important component of these lipids are alkylglycerols
639 (AKG) a class of ether lipids that constitute about 1% of total milk lipids. Roszer *et al.*
640 recently reported that AKG maintain beige adipose tissue (BeAT) in infants and delay the
641 transformation of BeAT into white adipose tissue in mice, thereby protecting against obesity.
642 They further report that breast milk AKGs are metabolized by adipose tissue macrophages to

form platelet-activating factor (PAF), which ultimately activates IL-6/STAT3 signaling in adipocytes and triggers BeAT development in the infant. This study suggests that lack of AKG intake in infancy leads to premature loss of beige adipose tissue and increased fat accumulation and points to a role in immune cells in this process. Alkylglycerols can also be metabolized into alkyl and alkenyl phospholipids. Our group and others have identified that these class of phospholipids are critical for human health and are depleted in obesity, diabetes and cardiovascular disease.

Breast milk represents the most abundant source of alkylglycerols in the human diet. While we see up to 17-fold increase in alkyldiacylglycerols and alkenylphosphatidylethanolamine species at 6 months and 12 months in breastfed infants, we did not observe any associations of breastfeeding at 12 months with 4 years circulating lipids. However, early modulation of these lipids might have long term health effects which is still to be understood.

Effect of infant sex on lipids: There is evidence of sex-differences in the lipidome from late gestation onwards [49]. For example, cord plasma concentrations of total cholesterol, HDL and LDL are higher in girls than in boys [50], in keeping with our findings. We have previously identified sphingomyelin SM (d18:2/14:0) as the key lipid differentiating sex in adult cohorts [8, 27]. At birth, we did not find SM (d18:2/14:0) to be different between male and female babies. However, at age 4 years, SM (d18:2/14:0) was significantly associated with sex and was lower in boys relative to girls (10.42% lower in boys, $p = 5.53 \times 10^{-4}$). In adults, the differential activity of the fatty acid desaturase (FADS3) is responsible for the elevated levels of SM (d18:2/14:0) in women [27, 51]. Our results suggest that FADS3 starts exhibiting this differential activity by four years. Additionally, sex differences in circulating gonadotropin levels during the first few months of life is well documented [52-54]. More recently, a differential hypothalamic-pituitary-adrenal (HPA) axis activity in response to foetal glucocorticoid exposure has been recorded between sexes at birth [55]. Understanding

the sex differences in the lipidome at birth and early life could provide mechanistic insights into sex-differences in cardiometabolic diseases.

Strengths and Limitations

Major strengths of this study include repeated and extensive sampling in a large population-derived cohort at in pregnancy and early life with high quality, standardised meta-data.

The BIS cohort provides an opportunity to study the effects of environmental factors during early infancy. However, the lack of generalisability (less ethnically diverse, more affluent than the general population) may limit the understanding of outcomes in different ethnicities and socio-economic strata. It would be valuable to follow the children through adolescence to understand the long-term modulation of lipid metabolism and early markers of future health or disease outcomes. We are currently investigating these results in another large birth cohort, based in Singapore – the Growing Up in Singapore Towards a healthy Outcome (GUSTO) cohort to investigate whether similar changes are evident in a population of Asian ethnicity. In this study, plasma lipidomics was performed on serum (maternal and newborn time points) and plasma (six, twelve months and four years). Several studies have pointed out to minor differences in lipid levels of these two matrices, however, these differences do not show any associations with biological functions [16].

Conclusion

We report in-depth lipidomic profiling in both gestation and the earliest stages of life, thereby providing insight into the ontogeny of lipid metabolism through infancy and early childhood. Despite the limitations, the extensive lipidomic profiling gives us insight into perinatal and postnatal factors associated with lipid metabolism during the first four years of life. There is now growing evidence of foetal programming emphasising the profound and sustained impact of intrauterine and early life factors to foetal health and development of metabolic

diseases in later life. Understanding the baseline characteristics of lipid metabolism at birth and throughout early years provide a resource for further studies to elucidate the clinical implications. The datasets resulting from this study now provide a rich resource to further investigate the relationship between lipid metabolism and health outcomes during early life. Children within this study are continuing to be followed and future outcome data will build on these initial early life findings.

Data availability

Due to the consent obtained during the recruitment process, it is not possible to make all data publicly available. Access to BIS data including all data used in this paper may be requested through the BIS Steering Committee by contacting the corresponding author. Requests to access cohort data are considered on scientific and ethical grounds and, if approved, provided under collaborative research agreements. Deidentified cohort data can be provided in Stata or CSV format. All statistical methods used are referenced within the methods section. Additional project information, including cohort data description and access procedures, is available at the project's website <https://www.barwoninfantstudy.org.au>.

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Conflict of interest

The authors declare no conflict of interest.

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Figure 2 - figure supplement 2 | The correlation between regression coefficients of each lipid species associated with gestational age (x-axis) and lipid species associated with birth weight (y-axis) was examined.

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876 **Figure 2 – figure supplement 3| a**, Volcano plot showing newborn phospholipids associated
 877 with gestational age. **b**, Volcano plot showing newborn plasma phospholipids associated with
 878 birth weight.
 879 Open circles represent all phospholipids (PL) and lysophospholipid (LPL) species. Colored
 880 circles represent PL and LPL containing odd and branched chain fatty acids. Red circles
 881 represent non-significant associations, blue circles represent significant lipids ($p < 0.05$),

882 green circles represent 10 most significant lipids (lowest p-values)
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886 **Figure 3 – figure supplement 1 1| a**, The correlation between regression coefficients of each
887 lipid species associated with assisted vaginal delivery (x-axis) and lipid species associated
888 with unscheduled caesarean delivery (y-axis) was examined. **b**, the correlation between
889 regression coefficients of each lipid species associated with assisted vaginal delivery (x-axis)
890 and lipid species associated with scheduled caesarean delivery (y-axis) was examined.

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Figure 5 – figure supplement 1 | A heat map showing the mean concentration of each lipid class at each time point. Orange: highest value, teal: lowest value

Figure 5 – figure supplement 2| Time series clustering on lipid species identified 10 different lipid clusters. We utilised Dynamic Time Warping (DTW) distance as a dissimilarity measurement. We used the partitional clustering method with partitions around medoids (PAM) to cluster the lipid species. We identified 10 different lipid clusters, each of them showing unique lipid trajectories over the first four years of life. Different lipid categories are shown in different colours.

Legend for Supplementary File 1

Supplementary File 1A: MRM transitions and conditions for examined lipid species

Supplementary File 1B: Cohort characteristics

Supplementary File 1C: Association of gestational age, birth weight and gender with lipid species at birth

Supplementary File 1D: Association of gestational age, birth weight and gender with lipid classes at birth

Supplementary File 1E: Effect of mode of delivery and duration of labour on newborn cord serum lipid species

920 Supplementary File 1F: Effect of mode of delivery and duration of labour on newborn cord
921 serum lipid classes

922 Supplementary File 1G: Effect of mode of delivery and duration of labour on maternal serum
923 lipid species

924 Supplementary File 1H: Effect of mode of delivery and duration of labour on maternal serum
925 lipid classes

926 Supplementary File 1I: Association of pre-pregnancy BMI and gestational diabetes with
927 maternal lipid species at 28 weeks gestation and differences between maternal and newborn
928 lipidome

929 Supplementary File 1J: Association of pre-pregnancy BMI and gestational diabetes with
930 maternal lipid classes at 28 weeks gestation and differences between maternal and newborn
931 lipidome

932 Supplementary File 1K: Change in lipid species concentration at different time points

933 Supplementary File 1L: Change in lipid classes concentration at different time points

934 Supplementary File 1M: LC-PUFA cholesteryl ester (CE) trends at different time points

935 Supplementary File 1N: Association of breastfeeding with lipid species at 6 months and 12
936 months

937 Supplementary File 1O: Association of breastfeeding with lipid classes at 6 months and 12
938 months

939 Supplementary File 1P: Association of sex with lipid species at different time points

940 Supplementary File 1Q: Association of sex with lipid classes at different time points

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