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Longitudinal lipidomic profiles during pregnancy and associations with neonatal anthropometry: findings from a multiracial cohort

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Summary

Background Maternal lipidomic profiling offers promise for characterizing lipid metabolites during pregnancy, but longitudinal data are limited. This study aimed to examine associations of longitudinal lipidomic profiles during pregnancy with multiple neonatal anthropometry using data from a multiracial cohort.

Methods We measured untargeted plasma lipidome profiles among 321 pregnant women from the NICHD Fetal Growth Study-Singletons using plasma samples collected longitudinally during four study visits at gestational weeks (GW) 10–14, 15–26, 23–31, and 33–39, respectively. We evaluated individual lipidomic metabolites at each study visit in association with neonatal anthropometry. We also evaluated the associations longitudinally by constructing lipid networks using weighted correlation network analysis and common networks using consensus network analysis across four visits using linear mixed-effects models with the adjustment of false discover rate.

Findings Multiple triglycerides (TG) were positively associated with birth weight (BW), BW Z-score, length and head circumference, while some cholesteryl ester (CE), phosphatidylcholine (PC), sphingomyelines (SM), phosphatidylethanolamines (PE), and lysophosphatidylcholines (LPC 20:3) families were inversely associated with BW, length, abdominal and head circumference at different GWs. Longitudinal trajectories of TG, PC, and glucosylcermides (GlcCer) were associated with BW, and CE (18:2) with BW z-score, length, and sum of skinfolds (SS), while some PC and PE were significantly associated with abdominal and head circumference. Modules of TG at GW 10–14 and 15–26 mainly were associated with BW. At GW 33–39, two networks of LPC (20:3) and of PC, TG, and CE, showed inverse associations with abdominal circumference. Distinct trajectories within two consensus modules with changes in TG, CE, PC, and LPC showed significant differences in BW and length.

Interpretation The results demonstrated that longitudinal changes of TGs during early- and mid-pregnancy and changes of PC, LPC, and CE during late-pregnancy were significantly associated with neonatal anthropometry.

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Keywords: Lipidomics; Pregnancy; Neonatal anthropometry; Birthweight; Body length; Sum of skinfolds; Sum of body circumferences

Research in context

Evidence before this study

Previous studies have focused on examining the potential utility of circulatory lipidomic trajectories in predicting GDM and several neonatal outcomes. Yet, few studies examined the relationship between longitudinal lipidomic profiling across pregnancy and neonatal anthropometry. Most of these studies assessed lipidomics using single-time collected gestational period samples prior to or after GDM diagnosis, and as such are unable to capture the dynamic changes of lipids profiles in pregnancy. Furthermore, small sample size, different analytical platforms, cross-sectional design, residual confounding, and different fetal and neonatal growth measures may have contributed to the inconsistent findings of previous studies.

Added value of this study

In this prospective study of 321 pregnant women aged 18–40 years from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Fetal Growth Studies-Singleton cohort, we examined the longitudinal and prospective association of lipidomic profiles throughout pregnancy with neonatal anthropometrics, including birthweight, birthweight z score, birth length, sum of skinfolds, and sum of body circumferences. We found that longitudinal changes of plasma TGs during early- and mid-pregnancy and PC, LPC, and CE during late-pregnancy were significantly associated with neonatal anthropometry. These findings indicate that sensible gestational age of maternal lipids profiles relevant to neonatal anthropometrics may differ by lipids sub-types. To the best of our knowledge, this is by far the largest and most comprehensive study on the prospective association between lipidomic trajectories and neonatal anthropometry.

Implications of all the available evidence

With an in-depth coverage of lipidomic profiles, coupled with longitudinal samples across gestation and the application of appropriate analytical methods, our findings have the potential to stimulate further investigation for exploring underlying pathophysiological mechanisms during pregnancy and assessing their clinical impact on neonatal outcomes.

Introduction

Pregnancy undergoes systemic and dynamic physiological adaptations that are essential to meet the nutritional and metabolic demands of both the mother and the developing fetus.[1] Lipids, an important class of structurally and functionally distinct molecules,[2] play a critical role in early embryo development and subsequent fetal growth.[3,4] Maternal lipid metabolites are thought to be regulated in large part by a plethora of hormonal and metabolic pathways across gestation. To better understand the role of lipids on maternal and fetal health outcomes, it becomes critical to characterize distinct maternal lipidomic profiles and trajectories during pregnancy.[5,6]

Earlier population studies reported that alterations in maternal triglycerides (TG), phospholipid (PL) and lysophosphatidylcholines (LPC) profiles have been associated with gestational diabetes mellitus (GDM).[7] Advanced lipidomics technologies permit the high-throughput assessment of a large number of lipid species in human bio-samples. Recently, maternal lipidomic profiles in plasma or serum samples have been associated with GDM[7] and perinatal outcomes, such as intrauterine growth restriction, pre-term birth,[8] birth weight (BW),[9–11] small size-for-gestational age at birth (SGA),[12,13] and large size-for-gestational age at birth (LGA).[10]

Some studies reported that higher maternal levels of plasma glycerophospholipids and sphingolipids at 20 weeks of gestation were associated with small for gestational age newborns.[14] However, previous studies, mostly small, have assessed lipidomics using a single-time collected gestational period samples prior to or after GDM diagnosis,[15] and as such are unable to capture the dynamic changes of lipids profiles in pregnancy. Furthermore, different analytical platforms, cross-sectional design, residual confounding, and different fetal and neonatal growth measures may have also contributed to the inconsistent findings of previous studies.

Some longitudinal lipidomic data, albeit sparse, highlights the potential utility of circulatory lipidomic trajectories in predicting GDM and several neonatal outcomes.[7,16,17] Yet, few studies examined the relationship between longitudinal lipidomic profiling across pregnancy and neonatal anthropometry.

Leveraging the longitudinal lipidomic data in the *Eunice Shriver Kennedy* National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies-Singletons. Specifically, we aimed to examine the longitudinal and prospective association of lipidomic profiles throughout pregnancy with neonatal anthropometrics, including birthweight, birthweight z score, birth length, sum of skinfolds (SS), and abdominal and head circumferences. We also examined whether maternal physiological status (i.e., obesity and race/ethnicity, and infant sex) may modify the associations.

Methods

Study design and population

The *Eunice Kennedy Shriver* NICHD Fetal Growth Studies-Singleton cohort (2009–2013) consisted of 2802 generally healthy women (2334 non-obese and 468 obese women) with singleton pregnancies and aged 18–40 years at enrolment.[18,19] All women were enrolled between 8 weeks 0 days and 13 weeks 6 days of gestation at 12 clinical centres throughout the U.S. and were followed up throughout their pregnancies.[18,19] For participants to be eligible, ultrasound estimates of gestational age at enrolment were required to be consistent (±5–7 days) with gestational dating, calculated by the last menstrual period. Sampling and eligibility criteria are described in detail elsewhere.[18,19] Full human subjects' approval was obtained from all participating clinical and data coordinating centers and the NICHD's institutional review boards. The IRB approval number is NICHD (09-CH-N152). All study participants gave their written informed consent prior to enrolment. The NICHD Fetal Growth Studies-Singletons is registered with Clinicaltrials.gov, number NCT00912132.

In the nested case–control study, a total of 107 women with incident GDM were identified as cases and matched randomly at a ratio of 1:2 to non-GDM controls on age (±2 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or Asian/Pacific Islander), and gestational age at blood collection (±2 weeks) (Supplemental Table S1).[20,21] The screening or diagnosis of GDM was conducted according to standard clinical care, at an average gestational age of 27 weeks.[22] Finally, we included 321 women with plasma samples in our analysis (107 women with GDM and 214 women without GDM). The detailed study protocol is available in the supplementary file for further reference.

Laboratory assays of lipidomic profiling

In this prospective cohort study, maternal blood samples were longitudinally collected from each participant at four study visits during pregnancy: gestational weeks (GW) 8–13 (enrolment visit 0), 16–22 (visit 1), 24–29 (visit 2), and 34–37 (visit 4).[18,19] However, the actual time ranges for blood collection were GW 10–14, 15–26, 23–31, and 33–39, respectively. All biospecimens were processed immediately and stored at −80 °C before assay.[18,19] Only the blood sample at weeks 15–26 was collected after an overnight fast due to the practical difficulties for pregnant women.

Lipidome analysis was performed at the West Coast Metabolomics Center, University of California Davis Genome Center. Details of laboratory methods used to quantify lipids were presented elsewhere.[23,24] Briefly, plasma lipidome was quantified using high-throughput liquid chromatography quadruple time-of-flight mass spectrometry (LC-QTOF MS/MS).[23,24] Internal standards were used for the calibration of retention times.[23] Peak intensities across samples were corrected by a bioreclamation quality control plasma-based normalization method[23] and used as quantitative measure for statistical analyses. All assays had inter-assay and intra-assay coefficients of variation <10% and were performed without the knowledge of GDM status.

A total of 420 non-targeted lipid metabolites, including 328 annotated and 92 unannotated metabolites, were profiled at visits 0, 1, 2, and 4.[7] The annotated metabolites belonged to four major lipid categories: glycerolipids (n = 145), including monoglycerides (MG, n = 3), diglycerides (DG, n = 16), and TG (n = 126); glycerophospholipids (n = 116), including phosphatidylcholines (PC, n = 83), phosphatidylethanolamines (PE, n = 11) and LPC (n = 22); sphingolipids (n = 62), including sphingomyelins (SM, n = 34), ceramides (Cer, n = 15), glucosylceramides (GlcCer, n = 9), and lactosylceramides (LacCer, n = 4); and sterol lipids (n = 20), primarily cholesteryl esters (CE, n = 18).[7]

Neonatal anthropometry

As described previously,[25] 6 anthropometric indicators, including birth weight, birth weight Z-score, length, abdominal circumference, head circumference, and sum of skinfolds, were measured and included as main health outcomes in this study. Birthweight and gestational age at delivery were abstracted from neonatal medical records. Gestational age- and sex-specific birthweight z-scores were derived using a U.S. national reference.[26] Neonatal length and skinfolds were measured by trained, certified study personnel. Measurements were obtained prior to discharge, 12–24 h after delivery; when it was impossible to obtain measurements at birth because of NICU admission or neonatal complications, infants born very preterm (≤32 weeks) were measured at 32 completed weeks of gestation-corrected age; and infants born moderately preterm (33–36 weeks) were measured once stabilized. Measurements were obtained in duplicate and a third measurement was obtained if the difference between the first two measurements exceeded prespecified tolerances based on expected technical errors of measurement.[27–29] Two closest measurements were averaged. Neonatal length, i.e., distance from the soles of infant's feet to the top of head, was measured with the infant supine using an Infantometer (SECA 416 Infantometer). Skinfold measurements were taken on the right side of the infant's body at the abdominal flank, anterior thigh, subscapular, and triceps using a Lange Skinfold Caliper (Beta Technology, Inc., Santa Cruz, CA) and summed as an indicator for total adiposity.[30,31] Circumference measurements were taken on the neonatal head, chest, abdominal (at a level midway between the xiphoid process and the umbilicus), umbilical, and mid-upper arm and summed for body circumferences.[32]

Assessment of covariates

Information on participant demographics, lifestyle factors, and past medical history was collected through a self-reported questionnaire. *A priori* selection of covariates, including nulliparity (yes/no) and prepregnancy BMI (kg/m^2), were assessed at study enrolment. Given that cases were matched with controls within a certain range of maternal age (years) and gestational age at biospecimen collection (weeks), we also included these two matching variables as covariates in the analysis.

2.5 Statistical analysis

All analyses were conducted in R version 4.0.4 (Austria, Vienna) and SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). To understand the relationship between the maternal plasma lipidomic metabolites and neonatal anthropometry including birth weight, birth weight Z-score, fetal length, SS, head circumference and abdominal circumference, we conducted four main analyses.

Lipidomics data were log-transformed to correct skewness and the batch effects were adjusted using the empirical Bayes method.[33] The principal component analysis was performed using the *prcomp* function in R on the pooled data from all visits and lipid species were reordered by the loadings on the first principal component to make species with higher correlations placed closer on the temporal heatmaps (Fig. 1).[34,35] Z-score pattern of the reordered lipid species showed broad temporal changes during pregnancy. We applied weighted mixed linear effect model at each visit to examine the association between individual metabolites and neonatal anthropometry. Because women with GDM were overrepresented in the analytic sample with biomarkers, the sample was reweighted to represent the full cohort (e.g., in the reweighted sample 4% of women had GDM as opposed to 33% in the non-weighted sample). As described previously,[20,36] weights were created per subject by the inverse of her sampling probability.[25] Sampling probability of each non-GDM subject was calculated from a logistic regression in the full cohort, excluding GDM cases. The dependent variable of this regression was "being selected as a non-GDM control" versus" being non-GDM but not selected as a control", while the independent variables consisted of matching factors including age, race/ethnicity, and gestational week at blood collection. This logistic regression allowed us to determine the probability of being sampled for each non-GDM control participant. The resulting sampling weights were designed to address the representation of the non-GDM control group in our study.

The models are adjusted by mother's age (continuous), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander), education (higher than high school/not), pre-pregnancy BMI (continuous), gestational age at blood collection (continuous), nulliparous (yes/no), and baby's sex. We visualized results using the volcano plots. We then further stratified the dataset by the baby's sex and mother's prepregnancy BMI (smaller than 30 or not), respectively. Multiple testing was corrected using the Benjamini–Hochberg false discovery rate (FDR) procedure.[37]

To examine the longitudinal trajectories of individual metabolites and their association with neonatal anthropometry, we first categorized trajectories of each metabolite using a weighted latent-class model.[38] For each biomarker, we allowed the number of trajectory groups to be 2–5 and degree up to 2 and fit polynomial curves. The final model was selected based on BIC using Jeffery's scale.[38] We required that final selected models had ≥5% probability to be in each identified group. The between-group difference for each metabolite in neonatal anthropometry was then examined using a weighted multivariate linear regression model with robust standard errors, adjusting for the aforementioned confounders for the associations of individual metabolite.

We agnostically constructed lipid networks at each study visit using a weighted correlation network analysis (also known as a weighted gene co-expression network method in R: WGCNA package).[39] We first set the least number of metabolites in each cluster to be 15 and the least dissimilarity between clusters to be at least 0.25 (scale-free R^2 >0.75).[39] Metabolites with a similar pattern were regarded as a specific module and marked by a unique colour. For each visit, we then obtained the scores on each module as the expressions on the module's first principal component and examined the associations of each given module with neonatal anthropometry using the same weighted multivariate linear regression model adjusting for the aforementioned confounders.

We also identified common metabolite networks across four visits using a consensus network analysis. We calculated the scores to construct each consensus module across four visits and categorized the trajectories of each module using a weighted latent-class model.[38] For each module, we still allowed the maximum number of groups to be 5 and the highest order of the polynomial curve to be 2. The final models were selected based on BIC and Jefferey's scale. We required that final selected models had ≥5% probability of being in each identified group. We then fitted the linear mixed effect model for neonatal anthropometry versus the trajectory groups adjusting for the same confounders and adjusted p-values using FDR adjustment. Significant modules were then identified as those modules with adjusted p-values lower than 0.05.

Role of the funding source

The study sponsors had no role in study design, the collection, analysis and interpretation of data, the writing of the report, or the decision to submit the paper for publication. The corresponding author had full

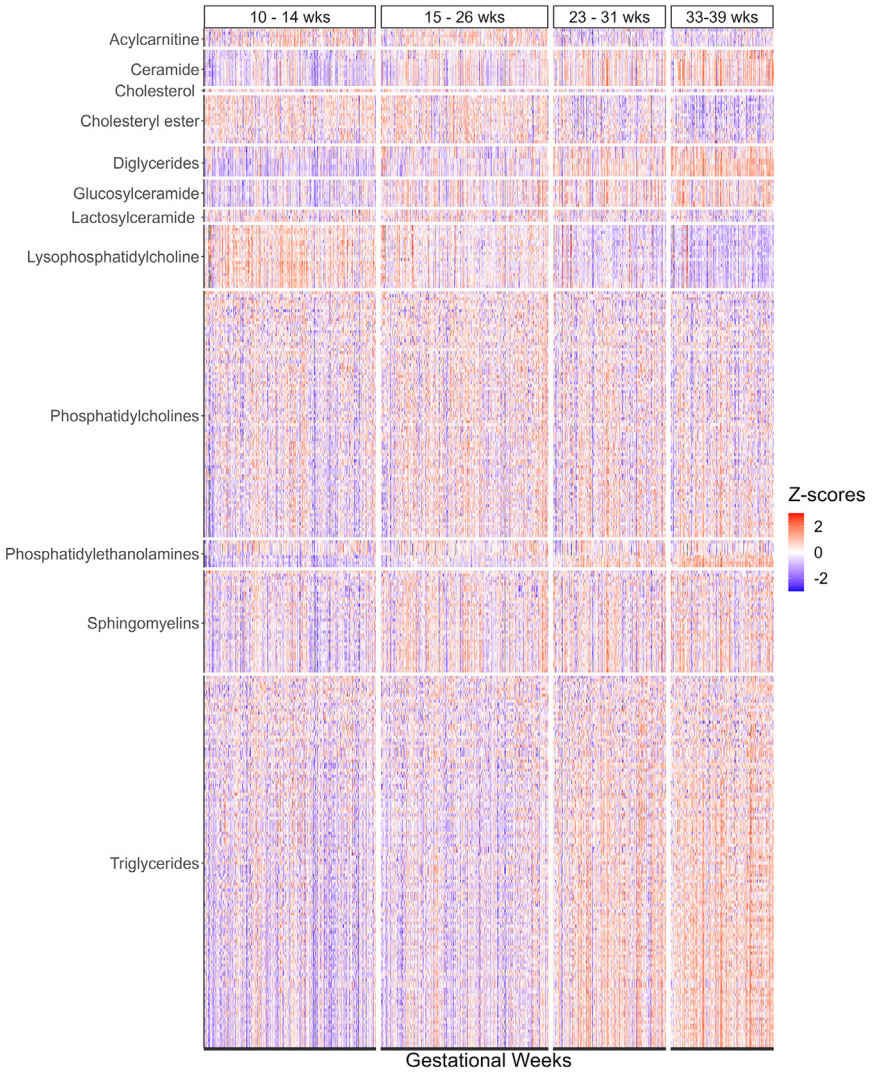

Fig. 1: **Z-score heatmap of 328 annotated lipidomic metabolites in the plasma collected at gestational weeks 10–14, 15–26, 23–31, and 33–39 among 321 pregnant women from the NICHD Fetal Growth Study-Singletons**. Data in heatmap is z-scored and is sorted by component 1 of the principal component analysis. Gestational weeks over the course of pregnancy are shown on the upper x-axis. The annotated lipid class name is shown on the y-axis. Each row represents the normalized intensities of a unique lipid. The features are color coded by row with red indicating high intensity and blue indicating low intensity.

access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

This study included a total of 328 annotated lipid metabolites measured at visits 0, 1, 2, and 4 over the entire gestational period and six neonatal anthropometrics as individual outcomes. Baseline characteristics of the 321 participants in the final analysis are shown as median (IQR) or frequency (percentages) (Supplemental Table S2). The mean age was 30.4 ± 5.5 y and the mean prepregnancy BMI was 26.5 ± 5.8 kg/m^2. Compared with non-obese women, obese women had lower education, lower gestational weight gain, and a higher risk of GDM.

As shown in Fig. 1, PLC and LC seemingly remained similar between each visit whereas others showed broad changes across time points during pregnancy. A major trend was that the majority of TG, DG, ceramide, GC, SM, and PE increased in abundance over time, whereas acylcarnitine and CE decreased in abundance.

Associations between lipid metabolites and neonatal biometry in each visit

The volcano plot showed significantly altered lipid species associated with neonatal anthropometry, including

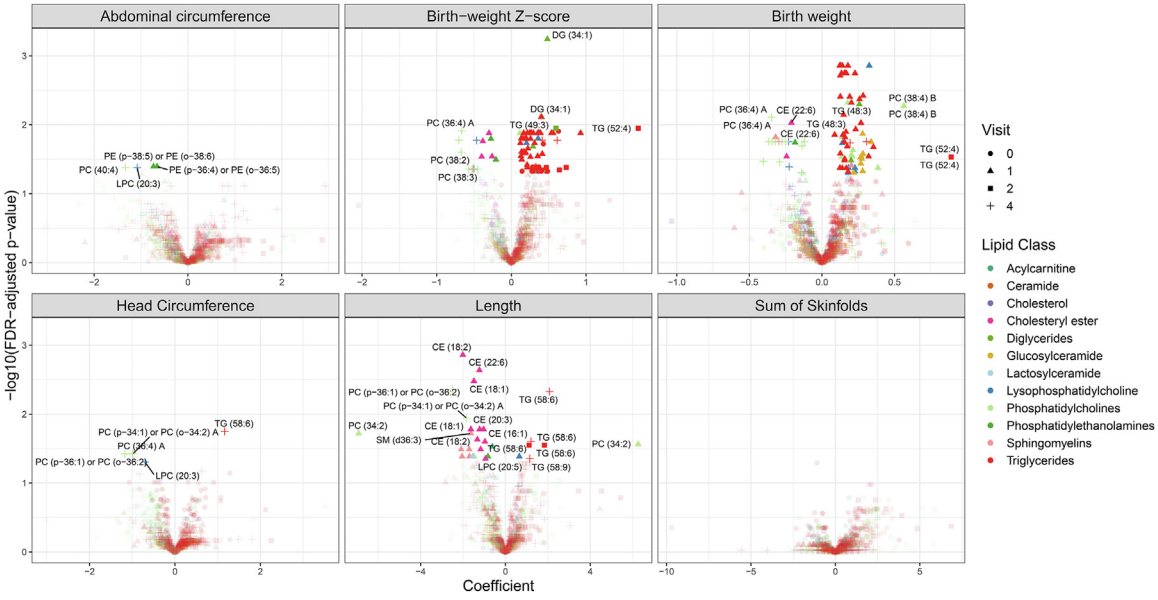

Fig. 2: **Volcano plots association between 328 annotated lipidomic metabolites and the neonatal anthropometry of interest for all visits**. Dot shape indicates visit. Dot color indicates the chemical family of the metabolites. Solid dots stand for significant metabolites while the transparent dots correspond to the non-significant metabolites. Annotations of the significant metabolites are shown in the figure as text.

BW, birthweight z-score, length, abdominal and head circumferences, and SS, respectively, at each of four visits (i.e. GW 10–14, 15–26, 23–31, and 33–39) (Fig. 2). The visit-specific Z-score plots of each metabolite in associations with each of six anthropometric measures were also shown in Supplemental Fig. S1–S6. The significant results were adjusted for confounding variables and corrected for multiple comparisons. None of lipidomic metabolites at each of the 4 visits was significantly associated with the sum of skinfolds.

We found that nine different TGs, mostly at GW 15–26, were positively associated with either BW or birthweight z-score; these TG species included TG (46:0), TG (48:1), TG (48:2), TG (48:3), TG (49:1), TG (49:2), TG (49:3), and TG (50:3) at GW 15–26 and TG (52:4) at GW 23–31 (Fig. 2 and Supplemental Fig. S1). TG (58:6) at GW 23–31 and TG (58:9) at GW 33–39 were positively associated with length and TG (58:6) at GW 33–39 was positively associated with length and head circumference (Fig. 2 and Supplemental Fig. S3 and S5. Neither abdominal circumference nor the sum of skinfolds was significantly associated with TG (Fig. 2 and Supplemental Fig. S4 and S6).

At GW 15–26, a small number of metabolites from DG (34:1), lysophosphatidylcholine (LPC) (17:1), and phosphatidylcholine (PC) (38:4) were positively associated with BW or birthweight z-score while four cholesteryl ester (CE) metabolites (18:1, 18:2, 18:3, and 22:6) were negatively associated with birthweight z-score (Fig. 2 and Supplemental Fig. S1 and S2). Seven CE metabolites (16:1, 18:1, 18:2, 18:3, 20:3, 20:4, and 22:6) and one sphingomyelin (SM) (d36:3) at GW 15–26 were negatively associated with length (Fig. 2 and Supplemental Fig. S3). Additionally, one PC (34:2) and one LPC (20:5) at GW 15–26 was positively associated with length while two phosphatidylethanolamines (PE) (p-36:4 or o-36:5 and p-38:5 or o-38:6) at GW 15–26 were negatively associated with abdominal circumference (Fig. 2 and Supplemental Fig. S3 and S4).

Some PC and LPC, mostly at GW 33–39, were negatively associated with neonatal anthropometric measures. Specifically, eight from PCs (30:1, 36:1, p-34:0 or o-34:1, p-36:1 or o-36:2, 36:4, 38:2, 38:3, 40:4), three from LPC (18:2, 20:2, and 20:3), and one from SM (d42:0) were negatively associated with BW or birthweight z-score (Fig. 2 and Supplemental Fig. S1 and S2).

At GW 33–39, one PC (40:4), and one LPC (20:3) were negatively associated with abdominal circumference while only three PC (p-34:1 or o-34:2, p-36:1 or o-36:2, and 36:4) and one LPC (20:3) were associated with head circumference (Fig. 2 and Supplemental Fig. S4 and S5). Also, two PCs (p-34:1 or o-34:2 and p-36:1 or o-36:2) were also significantly associated with length (Fig. 2 and Supplemental Fig. S3). Additionally, only one from SM (d42:0) other than PC, PE, and LPC at GW 33–39 was negatively associated with birthweight z-score (Fig. 2 and Supplemental Fig. S1).

Longitudinal associations between individual lipidomic metabolite trajectories and neonatal biometry

Longitudinally, each of the lipidomic metabolites had one or more distinct trajectory groups across gestation. We found that either BW or birthweight z-score was significantly associated with trajectories of several TG, PC, CE, or glucosylceramide (GlcCer) (Fig. 3). Two TG (58:1 and 53:1) and one PC (p-34:1 or o-34:2) were associated with both BW and birthweight z-score. Three GlcCer (d40:1, d42:1, and d42:2) were associated with BW while one CE (18:2) associated with birthweight z-score. A small number of CE, PC, and TG were longitudinally associated with the length or sum of skinfolds. Overall, 2 CE, 3 PC, and 3 TG were associated with length while 3 CE, 3 PC, 2 TG, and 1 PE were associated with the sum of skinfolds. In particular, the trajectories of CE (18:2) and TG (58:1) were significantly associated with both the length and sum of skinfolds. For abdominal and head circumferences, 2 PC, 1 LPC, 1 PE, and 1 TG were associated with abdominal circumference while 5 PC, 2 PE, 1 TG, and 1 lactosylceramide (LacCer) (d18:1/24:1) were associated with head circumference. Of note, trajectories of PC (p-40:6 or o-40:7) and PE (p-36:4) or PE (o-36:5) were significantly associated with both abdominal and head circumferences. Trajectories of one TG (58:1) were significantly associated with all neonatal anthropometry except head circumference.

Associations of lipidomic network modules at each visit with neonatal biometry

Of the 328 annotated metabolites, we identified 8, 7, 5, and 7 network modules at visit 0 (GW10-14), visit 1 (GW 15–26), visit 2 (GW 23–31), and visit 4 (GW 33–39), respectively (Fig. 4). The yellow module (clustering of module eigengenes was labelled with a unique colour in their names) rich in TGs at GW 10–14 is found to be significantly associated with birth weight Z-score. Of these TGs, TG (52:5) at GW 10–14 had the strongest association with birth weight Z-score. The pink and red modules at GW 15–26 were found to be significantly associated with birth weight. Most of the metabolites in the pink module were LPCs, of which LPC (17:1) at GW 15–26 contributed most to the significant associations with BW. The red module at GW 15–26 was also significantly associated with the birth weight Z-score. The significant metabolites with highest membership in the red module were TGs, including TG (48:1), TG (48:2), TG (49:1), TG (49:2), TG (49:0), TG (49:3), TG (46:1), TG (46:0), and TG (50:2). The blue and red

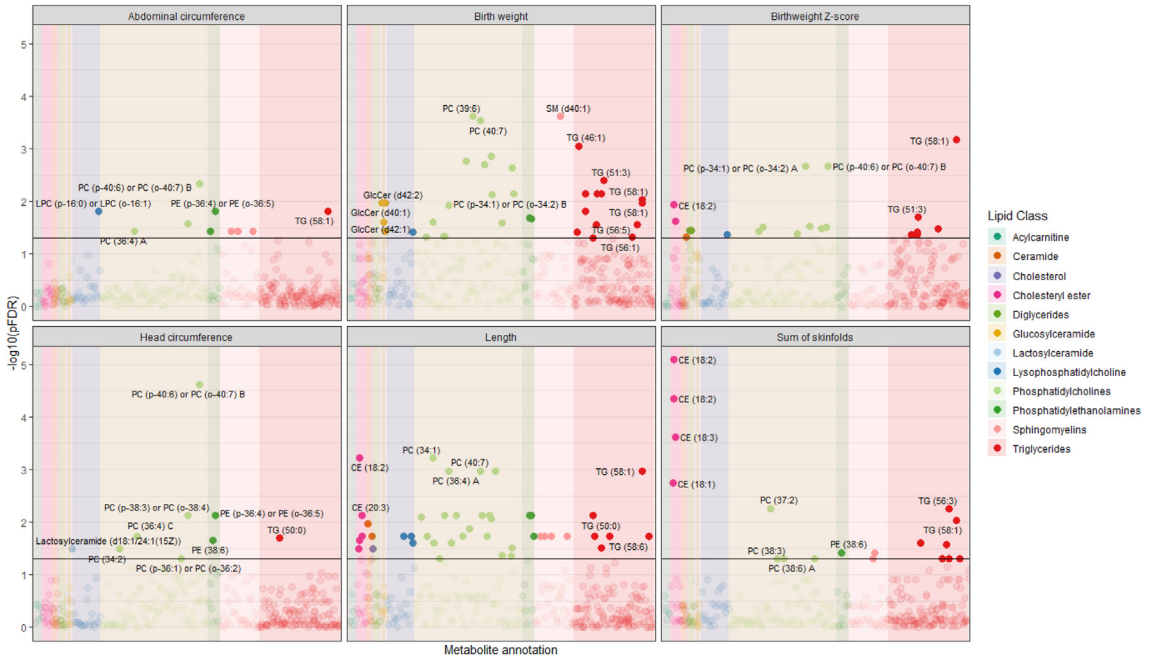

Fig. 3: **Manhattan plot of trajectory patterns of each metabolite in association with neonatal anthropometry**. FDR-adjusted p-values from mixed effect models adjusting for maternal age, race, education, preBMI, nulliparous and infant sex. The mixed effect models are fitted for the neonatal anthropometry with respect to the trajectory categories of each metabolite. p-values are from the type II ANOVA F-test. Dot color and background color indicate the chemical class of the metabolites. Metabolites with significant neonatal anthropometry–trajectory associations are shown as solid dots while the non-significant metabolites are shown as transparent dots.

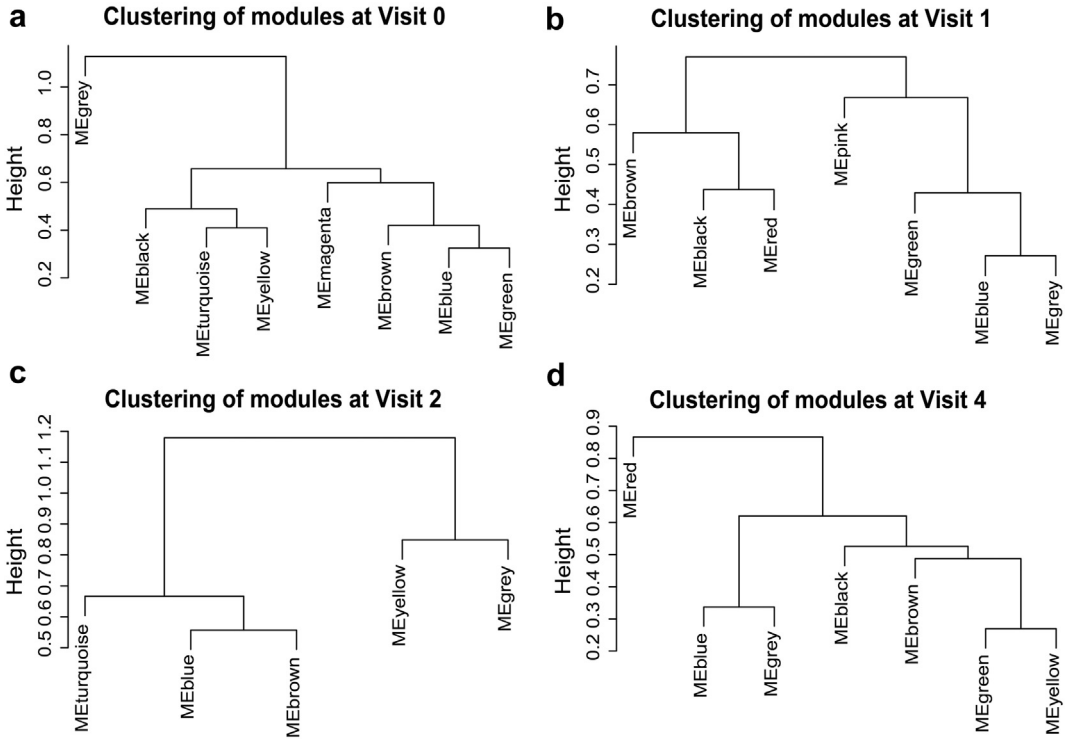

Fig. 4: **Tree plots of the visit-specific network modules.** Each module has at least 15 metabolites and the least dissimilarity between modules is 0.25. Note: "Height" on the y-axis represent the distance between clusters. Each leaf node represents a specific module.

modules at GW 33–39 were significantly associated with abdominal circumference. The blue module had 29 PC, 2 SM, 10 TG, 7 CE, and 2 DG. Only PC (40:4) in the blue module and LPC (20:3) in the red module contributed significantly to their associations with abdominal circumference.

Associations of consensus lipidomic network modules over gestation with neonatal biometry

The consensus network modules indicated that the main structure of the lipidomic network remained similar across the four visits. As shown in the correlations between network modules at each visit (y-axis) and the consensus network modules (x-axis), the 7 consensus networks preserved some network modules at each visit (Fig. 5). For example, the turquoise module of the consensus network contained most metabolites in blue modules at visit 0 and visit 1, the turquoise module at visit 2, and the grey module at visit 4. We found that the grey module was significantly associated with BW and length and the red module was significantly associated with infant length (Table 1). The red module included 17 lipid species (9 GluCer, 1 LacCer, and 7 PC). The grey module included 143 lipid species, mostly TG, PC and LacCer.

Discussion

In this prospective study, we investigated 328 lipidomic metabolites with discernible plasma levels longitudinally during gestation and found that some individual metabolites or networks, at each visit or across gestation, were consistently and significantly related to neonatal anthropometry, including birthweight, birth length, sum of skinfolds, and abdominal and head circumferences. In particular, we observed distinct trajectories mainly of TG, PC, CE, or LPC across gestation associated with neonatal anthropometric measures.

Given the fact that most earlier MS and NMR-based metabolomic studies only included limited numbers of individual glycerolipids in their platforms, we applied untargeted lipidomics approaches to allow for a high-throughput assessment of a large number of lipid species (126 TG, 16 DG, and 3 MG species) on maternal and fetal health outcomes. We found increased levels of 9 different TG species and DG (34:1), mostly at GW15-26, associated with large birth weight. TG (58:6) at mid and late pregnancy and TG (58:9) at late pregnancy were positively associated with length or head circumference. Of note, trajectories of one TG (58:1) identified in our study were significantly associated with all neonatal anthropometry except head circumference. Our findings are partly in line with the results of previous

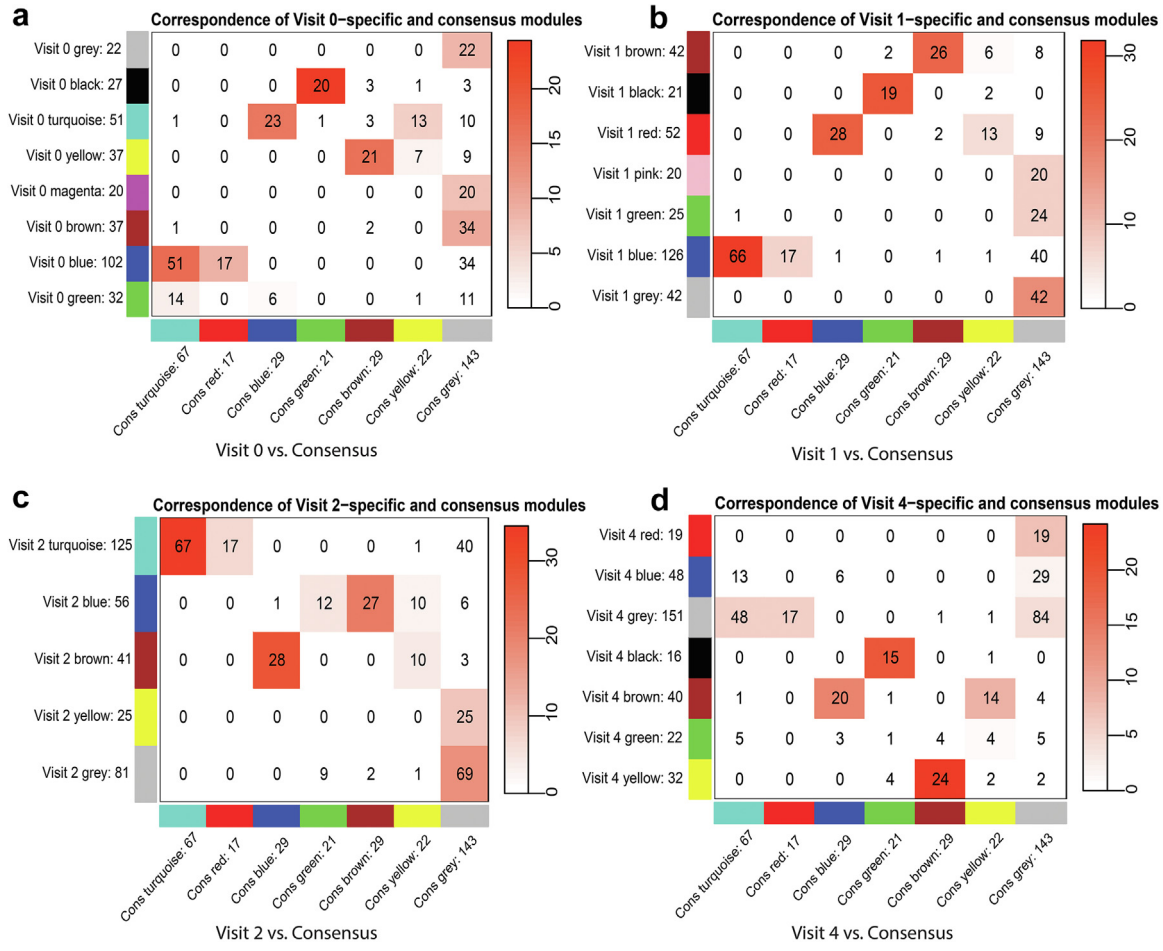

***Fig. 5:* Two-way table of metabolites belonging to different modules of each visit's network and the consensus network**. The background color shows the negative logarithm of p-values of the pairwise correlations. Redder color indicates more significant correlations.

studies,[9,10,12,14,40] although different TG species were found between our findings and previous studies.

Given the physiologic role of maternal hyperlipidemia in supplying both TG and cholesterol to the rapidly growing fetus, it is conceivable that unfavourable lipidomic profiles such as TG and CE may reflect the fetal derangements. Epidemiological studies have consistently shown that maternal hypertriglyceridemia was associated with increased offspring birth weight independently of maternal age, BMI, and GDM status.[41–44] Some studies reported that high maternal TG levels in early or mid-pregnancy were strongly associated with increased birth weight[44] or risk for LGA births.[45] In line with previous studies,[44,45] our findings showed similarly significant associations with TG even after correction for multiple testing and adjustment for confounders, including GDM status (data was not shown due to non-significance) and pre-pregnancy BMI. Physiologically, maternal TG cannot cross the placenta; thus, it must be hydrolyzed to fatty acids by placental lipases before it can be transported to the fetal circulation. High TG levels in maternal circulation may increase the mother-to-fetus gradient and facilitate placental glycerolipid transport to the embryo and fetal fat depots. Epidemiologically, some population characteristics such as ethnic ancestry, maternal BMI, weight gain during pregnancy, physical activity, smoking status, alcohol consumption, GDM, and glycemic control status may have influenced TG and DG levels and thus explain, in part, some inconsistent results in prior studies.[41–45]

In our study, levels of plasma PC, LPC, and CE, mostly during late pregnancy, were inversely associated with neonatal anthropometry independent of known maternal factors, including prepregnancy BMI, age, and race. Our findings using longitudinal lipidomic profiles were generally consistent with previous case–control studies assessing single-time maternal lipidomic profiles during pregnancy.[12,40] Horgan et al. identified

Consensus network modules			Neonatal outcome, Adjusted β (95% CI)[c]	p-value
			Birthweight, g	
Grey				
Reference group		Contrast Groups		
Trajectory class	Adjusted mean (95% CI)			
Group 3	3.28 (3.18, 3.38)	Group 4	−0.08 (0.08)	0.718
		Group 2	−0.18 (0.12)	0.404
		Group 1	−0.64 (0.16)	<0.001
Group 4	3.36 (3.22, 3.49)	Group 2	−0.11 (0.13)	0.851
		Group 1	−0.56 (0.17)	0.005
Group 2	3.46 (3.23, 3.69)	Group 1	−0.45 (0.19)	0.082
			Length, cm	
Grey				
Reference group		Contrast Groups		
Trajectory class	Adjusted mean (95% CI)			
Group 3	50.3 (49.8, 50.8)	Group 4	0.10 (0.36)	0.992
		Group 2	−0.75 (0.58)	0.563
		Group 1	−2.63 (0.73)	0.002
Group 4	50.2 (49.5, 50.8)	Group 2	−0.85 (0.64)	0.542
		Group 1	−2.74 (0.79)	0.003
Group 2	51.1 (49.9, 52.1)	Group 1	−1.87 (0.89)	0.149
Group 1	52.9 (51.5, 54.4)			
Red				
Trajectory Group		Contrast Groups		
Reference groups	Adjusted mean (95% CI)			
Group 2	50.1 (49.6, 50.6)	Group 1	−0.75 (0.36)	0.097
		Group 3	−2.62 (0.76)	0.002
Group 1	50.8 (50.2, 51.5)	Group 3	−1.87 (0.79)	0.047
Group 3	52.7 (51.2, 54.2)			

[a]Those with only one identified trajectory group are not included. [b]Each trajectory group was then associated with each neonatal outcome using a weighted multivariate regression, adjusting for age, pre-pregnancy BMI, race-ethnicity, education, and nulliparity. [c]The values colored red indicate that are nominal significant ($p < 0.05$).

Table 1: **Trajectories of consensus network modules across gestation with significant associations with neonatal birthweight and length.**[a,b]

nineteen metabolites at GW15 among 40 Australian women who developed SGA and 40 matched controls and reported higher levels of several lysoPC and PCs in SGA groups than controls. In another case–control study of mothers who delivered SGA (n = 20) and controls with appropriate gestational age babies (n = 20), Byeon et al. characterized the lipidome at GW 24–28 and found that only 20:4- lysophosphatidic acid (LPA) species was the most abundant lipid molecule (constituting about 45% of total LPA) decreased significantly in SGA mothers by 32% compared to control mothers.[40] Yet, these two studies had small sample sizes and assessed lipidomics using one single time collected samples, which cannot powerfully and fully capture the dynamic changes of lipids profiles in pregnancy.

Our lipidomics study also shows evidence of altered glycerophospholipids (GPL) and sphingolipid pathways associated with neonatal anthropometries. Our findings are partly in line with the results of previous studies. Although different GPL and SL metabolites were found between our findings and previous studies, several are involved in similar pathways and cellular processes. In a prospective study of 976 Dutch pregnant women and their children, Voerman and colleagues observed several individual maternal phospholipids during early pregnancy significantly associated with femur length and head circumference in the third trimester and weight and head circumference at birth.[11] Morillon et al. performed a lipidomics analysis in a case–control study of 40 SGA cases and 40 controls and showed an association between elevated maternal levels of GPL at GW 20 and higher risk of SGA.[14] It is evident, however, majority of prior studies lacked longitudinal lipidomic measurements across gestation to comprehensively characterize time-integrated lipidomic profiling during pregnancy. Their discrepant findings may be due to differences in sample size, timing of sample collection, sample types, analytical platforms and coverage, as well as characteristics of the study population.

The maternal–fetal transport mechanisms for lipids and cholesterol appear complex and are far from being understood. Phospholipids are the main lipid class

present in biological bilayers, such as cell membranes, and are involved in inflammation, apoptosis, and storage and breakdown of lipids for energy.[3] Physiological differences in these lipids could contribute to disruptions in multiple cellular processes involving the growth and development of embryos.[3,4] Alternatively, interindividual differences in some but not all lipids could be interpreted as resulting from an inadequate transplacental supply of the acid or its precursor coupled with a lack of the fetal enzymes necessary for the synthesis of these fatty acids.[4,46]

The present study has several strengths. We had longitudinal measurements of lipidomic biomarker levels across four time points throughout pregnancy, which allowed us to examine the temporal relationship between longitudinal changes in maternal lipidomic profiles and neonatal anthropometry. Specifically, our results highlight the significant associations between trajectory changes in TG, PC, LPC, and CE and multiple neonatal anthropometric measures. These significant associations persisted after adjustment for confounding variables and correction for multiple comparisons. To the best of our knowledge, this is by far the largest and most comprehensive study on the prospective association between lipidomic trajectories and neonatal anthropometry. Also, we applied network analyses for handling a group of highly correlated biomarkers. Additionally, the network of lipid biomarkers at either one visit or across gestation has been explored in the present study.

However, some potential limitations need to be acknowledged. First, even though ours is the largest thus far with longitudinal lipidomics measurement in pregnancy, the relatively small sample size limited our ability to address racial/ethnic disparities in the relationship between lipidomics and neonatal anthropometry. Second, due to the relatively moderate sample size, we were not able to evaluate more clinically relevant outcomes, such as LBW, SGA, and LGA. Meanwhile, the case–control design might affect the analyses for outcomes that were not the original target outcomes, even though a reweighted approach was used to represent the whole cohort population, which also limits the generalization to the general population. Third, concomitant changes in metabolites other than lipid species such as branched-chain and aromatic amino acids, non-esterified fatty acids, and phosphatidylcholines, might induce collinearity and further complicate the associations of lipidomics, which is difficult to disentangle especially when they might be highly correlated or in the same biological pathways. Future systematic analyses integrating these metabolites are warranted. Last, even though we tried to adjust for major known confounders, given the observational nature of the present study, possible confounding effects from unmeasured confounders can not be completely ruled out in this study.

Conclusions

In conclusion, we found that longitudinal changes of plasma TGs during early- and mid-pregnancy and PC, LPC, and CE during late-pregnancy were significantly associated with neonatal anthropometry. These findings indicate that sensible gestational age of maternal lipids profiles relevant to neonatal anthropometrics may differ by lipids sub-types. With an in-depth coverage of lipidomic profiles, coupled with longitudinal samples across gestation and the application of appropriate analytical methods, our data serve not only to advance our physiological understanding but also to identify potentially useful biomarkers for clinical translation. Our study also suggests that distinct and dynamic lipidomic signatures during pregnancy may be clinically relevant and informative for monitoring fetal and neonatal growth. Future large longitudinal studies with an objective assessment of neonatal anthropometry are warranted confirm and expand our findings.

Contributors

YQ S: Conceptualization, Methodology, Investigation, Data Curation, Writing–Original draft, Writing—Review & Editing. RJ L: Software, Formal analysis, Data curation. GQ Y: Investigation, Writing—Reviewing and Editing. ML R: Investigation, Writing—Reviewing and Editing. LW C: Investigation, Writing—Reviewing and Editing. YY Z: Investigation, Writing—Reviewing and Editing. O F: Investigation, Writing—Reviewing and Editing. MY T: Methodology, Investigation, Writing—Reviewing and Editing. Z C: Data curation, Methodology, Formal analysis, Writing—Reviewing and Editing. CL Z: Funding, Conceptualization, Methodology, Investigation, Data curation, Writing—Reviewing and Editing, Co-Principal Investigator for the overall Fetal Growth Study, Study Supervision. Both YQ S and RJ L have full access to the raw data. YQ S reviewed the dataset and code, and repeated the data analysis. All authors read and approved the final version of the manuscript.

Data sharing statement

The lipidomics data and phenotype data are not publicly available because they contain information that could compromise research participant privacy/consent. However, the data that support the findings of this study and the code used for all the models are available from the corresponding author upon reasonable request with a signed data access agreement.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104881.

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