**Placental polar lipid composition is associated with placental gene expression and neonatal body composition**

Olaf Uhl1\*, Rohan M Lewis2\*, Birgit Hirschmugl3,4, Sarah Crozier6,7, Hazel Inskip6,8, Antonio Gazquez1,5, Nicholas C Harvey6,8, Cyrus Cooper6,8, Gernot Desoye3, Berthold Koletzko1, Christian Wadsack3,4, Hans Demmelmair1,Keith M Godfrey6,8

\*Olaf Uhl and Rohan M Lewis contributed equally and share the first authorship

1 Department of Paediatrics, Dr von Hauner Children´s Hospital, University Hospitals, Ludwig-Maximilians-Universität Munich, Germany

2 University of Southampton, Faculty of Medicine

3 Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria

4 BioTechMed-Graz, Graz, Austria

5 University of Murcia, Department of Physiology, Murcia, Spain

6 MRC Lifecourse Epidemiology Unit, University of Southampton, UK

7NIHR Applied Research Collaboration Wessex, Southampton Science Park,

8 NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton

Corresponding author: Rohan M Lewis, University of Southampton Faculty of Medicine, MP887, IDS building, Southampton General Hospital, Southampton SO16 6UYD, rohan.lewis@soton.ac.uk

Olaf Uhl1\*, present address [**Olaf\_Uhl@web.de**](mailto:Olaf_Uhl@web.de)

Rohan M Lewis2\*, **rohan.lewis@soton.ac.uk**

Birgit Hirschmugl3,4, ***birgit.hirschmugl@medunigraz.at***

Sarah Crozier6,7, [***src@mrc.soton.ac.uk***](mailto:src@mrc.soton.ac.uk)

Hazel Inskip6,8, ***hmi@mrc.soton.ac.uk***

Antonio Gazquez1,5, ***antonio.gazquez@um.es***

Nicholas C Harvey6,8, ***nch@mrc.soton.ac.uk***

Cyrus Cooper6,8, ***cc@mrc.soton.ac.uk***

Gernot Desoye3, ***gernot.desoye@medunigraz.at***

Berthold Koletzko1, Berthold.Koletzko@med.uni-muenchen.de

Christian Wadsack3,4 ***christian.wadsack@medunigraz.at***

Hans Demmelmair1 ***Hans.Demmelmair@med.uni-muenchen.de***

Keith M Godfrey6,8 ***kmg@mrc.soton.ac.uk***

Declarations of interest: none

**Abstract**

The polar-lipid composition of the placenta reflects its cellular heterogeneity and metabolism. This study explored relationships between placental polar-lipid composition, gene expression and neonatal body composition.

Placental tissue and maternal and offspring data were collected in the Southampton Women’s Survey. Lipid and RNA were extracted from placental tissue and polar lipids measured by mass spectrometry, while gene expression was assessed using the nCounter analysis platform. Principal component analysis was used to identify patterns within placental lipid composition and these were correlated with neonatal body composition and placental gene expression.

In the analysis of placental lipids, the first three principal components explained 19.1%, 12.7% and 8.0% of variation in placental lipid composition, respectively. Principal component 2 was characterised by high principal component scores for acyl-alkyl-glycerophosphatidylcholines and lipid species containing DHA. Principal component 2 was associated with placental weight and neonatal lean mass; this component was associated with gene expression of *APOE*, *PLIN2*, *FATP2*, *FABP4*, *LEP*, *G0S2*, *PNPLA2* and *SRB1*. Principal components 1 and 3 were not related to birth outcomes but they were associated with the gene expression of lipid related genes. Principal component 1 was associated with expression of *LEP*, *APOE*, *FATP2* and *ACAT2*. Principal component 3 was associated with expression of *PLIN2*, *PLIN3* and *PNPLA2*.

This study demonstrates that placentas of different sizes have specific differences in polar-lipid composition and related gene expression. These differences in lipid composition were associated with birth weight and neonatal lean mass, suggesting that placental lipid composition may influence prenatal lean mass accretion.

***Keywords*** Lipidomics, Phospholipids, Pregnancy, Placenta, Gene expression

**Introduction**

Placental function and substrate supply are primary determinants of fetal development and have consequences for health during pregnancy and across the life course [1,2]. Polar lipid composition of the placenta may reflect multiple factors including maternal fatty acid supply, placental metabolism and placental cellular composition. These factors could affect placental function and thus the intrauterine environment and fetal development.

Polar lipids include different classes of phospholipids, sphingolipids found within the membranes and carnitines involved in lipid metabolism [3]. These lipids play important roles in the structure and function of lipid membranes. As specific lipids may be concentrated within lipid microdomains (e.g. sphingomyelins in lipid rafts [4]), within specific organelles or cell types their relative abundance may not reflect their biological importance to placental function. Fatty acids taken up by the placenta from maternal plasma may be incorporated into placental lipid pools or transported to the fetus [5,6]. Phospholipids are the major lipid pool within the placenta and there is evidence of selective partitioning of fatty acids into different placental lipid pools [7,8].

Different placental cell populations and subcellular membrane fractions have different lipid compositions which all contribute to what is measured in the lipid extracted from a tissue [9]. Changes in whole placental lipid composition may be explained by differences in cellular composition of the placenta, changes in placental lipid metabolism or altered maternal supply. The regulation of placental lipid metabolism within the placenta is not well understood, but there is evidence that it is altered in obese mothers [10,11].

This study explores how placental polar lipid composition and lipid-associated gene expression are related to birth outcomes, specifically placental weight and birthweight and neonatal body composition.

**Methods**

The study was conducted according to the guidelines of the Declaration of Helsinki, and the Southampton and South West Hampshire Research Ethics Committee approved all procedures (276/97, 307/97, 089/99, 153/99, 005/03/t, 06/Q1702/104). Written informed consent was obtained from all participating women.

***Maternal measurements***

We used data and samples from the Southampton Women’s Survey (SWS), a prospective cohort study of initially non-pregnant women aged 20-34 living in the city of Southampton, UK [12]. Women were recruited via their General Practitioners; assessments of lifestyle, diet and anthropometry were performed by trained research nurses at study entry; for those who later became pregnant the assessments were repeated in early (11 weeks) and late (34 weeks) gestation. Subscapular skinfold thicknesses were measured to the nearest 0.1 mm in triplicate using Harpenden skinfold callipers [13]. Of the 3158 liveborn singleton births in the SWS, of these 99 were collected rapidly after delivery allowing for RNA isolation as well as having neonatal body composition data assessed using dual-energy X-ray absorptiometry (DXA) and these were selected for this study.

***Placental samples***

Placentas were collected from SWS term pregnancies within 30 minutes of delivery. Placental weight was measured after removing blood clots, cutting the umbilical cord flush with its insertion into the placenta, trimming away surrounding membranes and removing the amnion from the basal plate [14]. Villous tissue samples were selected using a random sampling method and stored at -80°C. Gene and lipid analysis was performed the same villous tissue sample from each placenta.

***Offspring body composition measurement in SWS***

Within 3 weeks of birth, a subset of infants underwent an assessment of body composition by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L instrument (GE Corp). The total X-ray dose for the whole-body scans was approximately 10.5 microsieverts (paediatric scan mode), which is equivalent to approximately 1–2 days background radiation. All scan results were checked independently by two trained operators, and agreement was reached as to their acceptability; scans showing unacceptable movement artefact were excluded. Lean and fat mass were derived from the whole-body scan through the use of paediatric software (Hologic Inc.) [15].

***Polar lipid analyses of placental tissue***

Placenta samples were analysed at the Department of Paediatrics, LMU Munich, Germany. Placental tissue was homogenised and the lipids were extracted according to the method of Folch with CHCl3/MeOH, including dipentadecanoyl phosphatidylcholine (15:0/15:0) as the internal standard to correct for effects during sample extraction [16]. The extract was diluted with methanol, containing lysophosphatidylcholine LPC(13:0), phosphatidylcholine PC(14:0/14:0), D3-Carnitine(2:0), D3-Carnitine (8:0) and D3-Carnitine (16:0) as internal standards and ammonium-acetate. Extracts were analysed by flow-injection mass spectrometry using a triple quadrupole mass spectrometer (QTRAP4000, Sciex, Darmstadt, Germany) with an electrospray ionisation (ESI) source in positive ionisation mode as previously described [17]. The mass spectrometer was coupled to an HPLC system (Agilent, Waldbronn, Germany). The entire analytical process was post-processed by Analyst (ver. 1.5.1), and the isotopomer correction was applied in R (ver. 3. 0.1).

The analysis comprised acylcarnitines (Carn), lysophosphatidylcholines (Lyso.PC), diacyl-phosphatidylcholines (PC.aa), acyl-alkyl-phosphatidylcholines (PC.ae) and sphingomyelines (SM). As a point to note, the analytical technique applied here is not capable of determining the position of the double bonds and the distribution of carbon atoms between fatty acid side chains. The polar lipids nomenclature is XX:Y where, X is the combined length of the carbon chains, Y is the number of double bonds.

The analysis of polar lipids is a screening method that comprised 484 different mass transitions; non-polar lipids are not measured using this methodology. The samples were measured in two batches with 76 samples in batch 1 and 32 in batch 2. The first step of quality control was to check the precision of quality control samples (6 aliquoted plasma samples in each batch). All analytes with more than 20% intra-batch precision and more than 30% inter-batch precision were removed. From the remaining 82 metabolites, 3 were rarely present in the samples and were removed. Blank samples were used to identify contaminants and four metabolites where the blank values represented > 10% of the sample average were removed. The final metabolite number was 75. The primary fatty acids associated with each lipid species are listed in supplementary data table S1. The metabolite concentrations of the samples were corrected for the internal standard and are given in µmol/kg tissue or expressed as a % of the 75 measured metabolites in each sample.

***RNA isolation, quality assessment and analysis by the nCounter system***

Analysis of placental gene expression was performed at the Medical University of Graz. Frozen placental villous tissue (50 – 100 mg) was homogenised in RLT lysis buffer (Qiagen, Hilden, Germany) by using Precellys ceramic kit (Peqlab, Erlangen, Germany) and the MagNA lyser system (Roche, Basel, Switzerland). RNA was isolated from placenta tissue homogenates by RNeasy mini kit (Qiagen, Hilden, Germany) following the protocol of the manufacturer. The RNA quality control was performed on a 2100 Bioanalyzer Instrument (Agilent Technologies, Santa Clara, USA) and only samples with an RNA integrity number (RIN) above 7.0 were considered for analysis [18]. Gene expression analysis was performed on the nCounter analysis platform (NanoString Technologies, Seattle, WA) according to manufacturer’s instruction. For the analysis, RNA (400 ng) was probed with a custom Code Set containing 30 genes selected for their association with lipid transport and metabolism and, of these, four genes were below the limit of detection and were not analysed further (supplementary table S2). The analysis was performed using the nSolver 2.0 Analysis Software (NanoString Technologies, Seattle, WA) [19]. The 6 control genes were *ANGEL1* (NM\_015305.3), *OAZ1* (NM\_004152.2), *PPIA* (NM\_02113 0.2), *RPL30* (NM\_000989.2,) *TBP* (NM\_001172085.1), *WDR45L* (NM\_019613.3).

***Statistics***

Summary data are presented as mean and standard deviation (SD) for normally distributed variables and median and interquartile range (IQR) for non-normally distributed variables.

Principal component analysis was performed on the lipid proportion variables [20]. Maternal and neonatal variables which were not normally distributed were transformed to normality using Fisher-Yates normal scores [21]. Percentage lipids, gene expression and principal components were similarly transformed using Fisher-Yates normal scores. Relationships between maternal and neonatal body composition variables and lipid principal component scores were analysed using linear regression models adjusted for sex and gestation with resulting beta coefficients describing the change in body composition in SDs per SD change in lipid principal component scores; the same approach was used for body composition variables and percentage placental lipid species. Relationships between lipid principal component scores and gene expression were analysed using linear regression models adjusted for sex and gestation with resulting beta coefficients describing change in lipid principal component scores in SDs per SD change in gene expression; the same approach was used for percentage lipid species and gene expression [21]. Data were analysed using Stata version 14.1 (StataCorp, Texas, USA). A P-value of < 0.05 was considered to be significant but for the individual comparisons between lipids and postnatal outcomes we have chosen to focus the discussion on those findings reaching the P < 0.01 level of significance. For the individual comparisons between gene expression and lipid composition we have chosen to focus the discussion on those findings reaching a lower P value of < 0.001.

**Results**

***Cohort data***

Maternal and neonatal data for the 99 participating mother-child pairs studied are provided in Table 1.

***Lipidomic data***

The lipidomic analysis provided information on 75 polar lipid species including Carn, PC.aa, PC.ae, Lyso.PC and SM in samples from the 99 placentas. The full list of species measured, and summary values are provided in supplementary table S3. All analyses were performed on the % data. To reduce the complexity of the data principal component analysis was performed on the percentage lipid data. The first 3 principal components explained 19.1%, 12.8% and 8.1% of the variation in the data respectively.

Principal component 1 was characterised by lipids containing arachidonic acid (20:4 n-6, ARA), docosahexaenoic acid (22:6 n-3, DHA) and eicosapentaenoic acid (20:5 n-3, EPA) having low coefficients while lipids with high coefficients contained a preponderance of PC.aa and SM. Principal component 2 was characterised by high coefficients for PC.ae as well as lipid species containing DHA and ARA. Principal component 3 was characterised by lipids containing ARA being concentrated in the low-coefficient lipids.

Coefficients for the first three principal components can be found in supplementary table S4.

***Placental lipid content (%), maternal factors, neonatal measures***

There were no notable relationships between lipid principal component scores and maternal pre-pregnancy body composition (Table 2).

Higher placental weight, birthweight and neonatal lean mass were associated with higher lipid principal component 2 scores (Table 2).

Percentages of multiple lipids were related to birth outcomes and all associations can be seen in supplementary table S5. Focusing on those associations at the p = 0.01 level of significance, 9 placental lipids were related to placental weight (positive association: PC.aa.C34.1, SM.a.C34.2, PC.aa.C36.1, SM.a.C36.2, SM.a.C35.2, Lyso.PC.a.C18.0; negative association: PC.ae.C40.0, PC.aa.C38.5, PC.aa.C34.4), 5 lipids were related to birthweight (positive: PC.aa.C36.1, PC.aa.C34.1, PC.aa.C36.2; negative: PC.ae.C40.0, PC.aa.C38.5), 4 lipids were related to neonatal lean mass (positive: PC.aa.C34.1, PC.aa.C36.1, negative: PC.ae.C40.0, PC.aa.C38.5) and two lipids were related to neonatal fat mass by DXA (PC.aa.C34:1, PC.aa.C36.1) (Table 3).

***Placental lipid content and gene expression***

Expression of 26 of 30 target genes was successfully measured in the placenta. Placental lipid principal component 1 score was associated with gene expression for *LEP* and *APOE* and negatively with *FATP2* and *ACAT2* (Table 4). Placental lipid principal component 2 score was associated with the expression of 8 genes including *APOE*, *PLIN2*, *FABP4* and *LEP* (Table 4). Principal component 3 was negatively related to the lipid droplet and triglyceride associated genes *PLIN2*, *PLIN3* and *PNPLA2* (Table 4).

The expression of multiple genes were related to lipids and all associations can be seen in supplementary table S6. Genes that were related to two or more individual lipid species at P ≤ 0.001 are presented in Table 5. Of note, FATP2 was related to nine polar lipid species and *APOE* and *LEP* were each related to five polar lipid species at P ≤ 0.001.

**Discussion**

This study found relationships between placental lipid composition and placental weight, birth weight and neonatal lean mass. As fetal growth is a product of placental function, elucidating the role of these lipids in the placenta may highlight placental determinants of fetal growth. Furthermore, the relationships between placental lipid composition and the expression of lipid-related genes suggest underlying regulation of lipid composition and gene expression.

The lipid composition of the placenta, as represented by principal component 2, was related to placental weight. This observation suggests that a larger placenta is not simply a scaled-up version of a smaller placenta (or vice versa), as, if this were the case, while there would be more lipid in a larger placenta, the lipid composition would be the same. Furthermore, principal component 2 was related to birth weight and neonatal lean mass suggesting that whatever underlies the difference in lipid composition is either a determinant of placental function and birth outcome or is determined by a common factor.

The mechanism underlying the relationship between lipids associated with principal component 2, or of individual lipids, and birth outcome is not obvious. A change in villous lipid composition could be explained by alterations in cellular composition, changes in lipid metabolism or changes in maternal lipid supply. The lipid species identified could also play critical roles as lipid precursors for signalling (both within or between cells) or in mediating metabolic flux of fatty acids through different lipid pools, determining their availability to the fetus. Identifying the distinct lipid signatures of isolated primary placental cell populations could help to determine whether or not the observed differences could reflect cellular heterogeneity. Looking at individual lipids and birthweight, it is interesting to note that PUFA containing lipids (e.g. PC38:5) have negative association while more saturated lipids (e.g. PC34:1, SM34:2) are positively associated. Lower levels of PUFA containing lipids in the placenta could represent either reduced maternal supply or increased transfer of PUFA containing lipids to the fetus.

Neonatal outcomes were associated with principal component 2, which had positive scores for PC.ae and with polyunsaturated fatty acids. Interestingly, these acyl-alkyl lipids tended to change together, and this may reflect a common biosynthetic pathway. These acyl-alky lipids may play specific roles within the cell, including in membrane trafficking, cell signalling and oxidative stress [22]. One study has looked at the molecular identification of phospholipids in the human term placenta and found that SM and phosphatidylcholine were distributed differently between stem and terminal villi [23].

The individual lipid species that were most strongly related to postnatal outcomes had a wide range of abundances, but their median abundance was similar to that of their respective lipid classes. Only one of these contained a polyunsaturated fatty acid, PC.aa.8\_5, which contains ARA.

Of note, a distinct distribution of PC.aa (16:0/20:4) coupled with ARA in terminal villi has been reported [9]. These arachidonic acid containing phospholipids are of particular interest in the placenta where together with DHA they make up around 40% of all phospholipids [7]. ARA and its metabolites (prostaglandins and leukotrienes) are considered as second messengers and do play a critical role in placental function, development of the fetus and parturition.

Neither principal component 1 or 3 were related to birth outcomes, but they did have clear relationships with placental gene expression. Principal component 1 was characterised by lipids containing ARA, DHA and EPA having low principal component coefficients, while lipids with high principal component coefficients contained a preponderance of PC.aa and SM. Principal component 3 was characterised by lipids containing ARA being concentrated in the low-coefficient lipids. Principal component 3 was characterised by lipids containing ARA being concentrated in the low scoring lipids.

The relationships between placental lipid composition and the expression of lipid-related genes were interesting and points to underlying regulatory relationships. Placental expression of genes including *LEP*, *APOE* and *FATP2* were associated with both principal components 1 and 2, while expression of *PLIN2* and *PNPLA2* were related to both principal components 2 and 3. In addition to the principal components, relationships were observed between gene expression and individual lipid species. As different patterns of association were observed, this suggests multiple regulatory pathways linking lipid composition and gene expression.

The lipids with the strongest relationships to gene expression presented in table 5 included a wide range of abundance but did not include any lipid species in the bottom 20% of abundance. For PC.aa and PC.ae, there was a tendency for those lipids in table 5 to be below the median abundance for that class. In contrast, the lysophospholipids and sphingomyelins in table 5 had similar median abundances to their respective classes. The lipids in table 5 also contained 3 out of the 6 measured DHA containing species but only 1 lipid of 8 measured species contained ARA. This suggests that there may be an enrichment of DHA containing lipid species associated with gene expression. However, further studies would be needed to confirm this.

Whether gene expression causes changes in lipid composition, lipid composition is regulating gene expression, or they are both regulated by a related factor cannot be determined from this data. However, these relationships highlight avenues for future investigation.

Recent studies have demonstrated associations between maternal obesity and the placental triglyceride content and phospholipid composition [7,10] as well as rates of placental beta-oxidation [11]. It is, therefore, interesting that this study found no overall relationship between maternal adiposity and polar lipid composition. A previous study has demonstrated associations between placental polar lipids and maternal obesity, and so this question requires further investigation [7].

A strength of this study is that it has investigated a large number of individual lipids and identified patterns of lipid expression through principal component analysis, and related these to outcomes. However, a challenge for translating these findings into health benefits is identifying what the patterns of lipid and gene expression relate to at a biological level. A limitation of this study is that the lipid and gene expression analysis was performed on whole placental tissue which contained multiple different cell types. However, as placental cell-type-specific lipidomic and transcriptomic signatures become available, this will facilitate the biological understanding of these findings and those of similar studies. Another limitation of the study is that a panel of genes was investigated rather than the whole transcriptome. However, using these 30 genes, which were selected based on their relevance in lipid homeostasis, the study was able to demonstrate clear associations between gene expression and the lipid principal components.

This study was designed to identify relationships within the population as a whole, but it should be noted that there may also be differences that occur at the extremes of the population that are not observed here. For instance, comparing pregnancies with very high BMI to those in the normal range may identify difference not seen here because this study does not include large numbers of pregnancies with extreme BMI.

This is a cross sectional study, so it is not possible to ascribe causality to the observations made, either between gene and lipid levels in the placenta or between placental factors and neonatal outcomes. For instance, fetal lean mass may secrete hormones that affect placental lipid composition or altered placental lipid composition may reflect functional differences which promote growth of fetal lean tissues. However, by demonstrating these associations this study provides a framework to study causal the relationships between gene expression and lipid composition or between placental lipid composition and fetal growth in the future.

This study indicates that there is an identifiable pattern of lipid composition within the placenta that related to birth weight. Understanding these features at the biological level will help explain variation within the association between placental and fetal weight and provide targets for intervention. Identifying the patterns of lipids related to good birth outcomes provides additional data which may help identify the underlying processes and provide targets for therapeutic interventions.

In conclusion, this study demonstrates that placentas of different sizes are not just bigger or smaller but have specific differences in polar lipid composition. The multiple associations between placental lipid composition and gene expression suggest underlying regulatory relationship. These differences may underlie differences in birth weight and neonatal lean mass, and further characterisation of these may provide a route to effective interventions in poorly growing fetuses.

**Acknowledgements:** This work has been financially supported in part by the European Union's Seventh Framework Programme (FP7/2007-2013), project EarlyNutrition (grant agreement n°289346) and the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG 322605). BK and KMG are supported by the European Union Erasmus+ Capacity-Building ENeASEA Project. BK is the Else Kröner-Seniorprofessor of Paediatrics at LMU supported by the Else Kröner-Fresenius-Foundation and LMU Munich. KMG is supported by the UK Medical Research Council (MC\_UU\_12011/4), the National Institute for Health Research (NIHR Senior Investigator (NF-SI-0515-10042) and the NIHR Southampton Biomedical Research Centre) and European Union's Seventh Framework Programme (FP7/2007-2013, projects EarlyNutrition and ODIN under grant agreements 289346 and 613977). BH was supported by anniversary fund of the Austrian National Bank (OeNB 18181). HMI is supported by the UK Medical Research Council (MC\_UU\_12011/4).

**References**

[1] G.J. Burton, A.L. Fowden, K.L. Thornburg, Placental Origins of Chronic Disease, Physiol Rev 96 (2016) 1509-1565. 10.1152/physrev.00029.2015.

[2] R.M. Lewis, J.K. Cleal, M.A. Hanson, Review: Placenta, evolution and lifelong health, Placenta 33 Suppl (2012) S28-32. 10.1016/j.placenta.2011.12.003.

[3] Polar Lipids: Biology, Chemistry, and Technology, Academic Press and AOCS Press2015.

[4] M.C. Giocondi, S. Boichot, T. Plenat, C.C. Le Grimellec, Structural diversity of sphingomyelin microdomains, Ultramicroscopy 100 (2004) 135-143. 10.1016/j.ultramic.2003.11.002.

[5] R.M. Lewis, C. Wadsack, G. Desoye, Placental fatty acid transfer, Curr Opin Clin Nutr Metab Care 21 (2018) 78-82. 10.1097/MCO.0000000000000443.

[6] S. Perazzolo, B. Hirschmugl, C. Wadsack, G. Desoye, R.M. Lewis, B.G. Sengers, The influence of placental metabolism on fatty acid transfer to the fetus, J Lipid Res 58 (2017) 443-454. 10.1194/jlr.P072355.

[7] O. Uhl, H. Demmelmair, M.T. Segura, J. Florido, R. Rueda, C. Campoy, B. Koletzko, Effects of obesity and gestational diabetes mellitus on placental phospholipids, Diabetes Res Clin Pract 109 (2015) 364-371. 10.1016/j.diabres.2015.05.032.

[8] O.C. Watkins, M.O. Islam, P. Selvam, R.A. Pillai, A. Cazenave-Gassiot, A.K. Bendt, N. Karnani, K.M. Godfrey, R.M. Lewis, M.R. Wenk, S.Y. Chan, Metabolism of 13C-Labeled Fatty Acids in Term Human Placental Explants by Liquid Chromatography-Mass Spectrometry, Endocrinology 160 (2019) 1394-1408. 10.1210/en.2018-01020.

[9] Y. Kobayashi, T. Hayasaka, M. Setou, H. Itoh, N. Kanayama, Comparison of phospholipid molecular species between terminal and stem villi of human term placenta by imaging mass spectrometry, Placenta 31 (2010) 245-248. 10.1016/j.placenta.2009.12.026.

[10] B. Hirschmugl, G. Desoye, P. Catalano, I. Klymiuk, H. Scharnagl, S. Payr, E. Kitzinger, C. Schliefsteiner, U. Lang, C. Wadsack, S. Hauguel-de Mouzon, Maternal obesity modulates intracellular lipid turnover in the human term placenta, Int J Obes (Lond) 41 (2017) 317-323. 10.1038/ijo.2016.188.

[11] V. Calabuig-Navarro, M. Haghiac, J. Minium, P. Glazebrook, G.C. Ranasinghe, C. Hoppel, S. Hauguel de-Mouzon, P. Catalano, P. O'Tierney-Ginn, Effect of Maternal Obesity on Placental Lipid Metabolism, Endocrinology 158 (2017) 2543-2555. 10.1210/en.2017-00152.

[12] H.M. Inskip, K.M. Godfrey, S.M. Robinson, C.M. Law, D.J. Barker, C. Cooper, S.W.S.S. Group, Cohort profile: The Southampton Women's Survey, Int J Epidemiol 35 (2006) 42-48. 10.1093/ije/dyi202.

[13] G. Harrison, E. Buskirk, Carter J, F. Johnston, T. Lohman, M. Pollock, Skinfold thicknesses and measurement technique, In Anthropometric standardization reference manual Human Kinetics Books, Champaign, Illinois, 1988, pp. 55-70.

[14] S.D. Leary, K.M. Godfrey, L.J. Greenaway, V.A. Davill, C.H. Fall, Contribution of the umbilical cord and membranes to untrimmed placental weight, Placenta 24 (2003) 276-278.

[15] N.C. Harvey, J.R. Poole, M.K. Javaid, E.M. Dennison, S. Robinson, H.M. Inskip, K.M. Godfrey, C. Cooper, A.A. Sayer, S.W.S.S. Group, Parental determinants of neonatal body composition, J Clin Endocrinol Metab 92 (2007) 523-526. 10.1210/jc.2006-0456.

[16] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J Biol Chem 226 (1957) 497-509.

[17] O. Uhl, M. Fleddermann, C. Hellmuth, H. Demmelmair, B. Koletzko, Phospholipid Species in Newborn and 4 Month Old Infants after Consumption of Different Formulas or Breast Milk, PloS one 11 (2016) e0162040. 10.1371/journal.pone.0162040.

[18] B. Hirschmugl, S. Crozier, N. Matthews, E. Kitzinger, I. Klymiuk, H.M. Inskip, N.C. Harvey, C. Cooper, C.P. Sibley, J. Glazier, C. Wadsack, K.M. Godfrey, G. Desoye, R.M. Lewis, Relation of placental alkaline phosphatase expression in human term placenta with maternal and offspring fat mass, Int J Obes (Lond) 42 (2018) 1202-1210. 10.1038/s41366-018-0136-8.

[19] M.H. Veldman-Jones, R. Brant, C. Rooney, C. Geh, H. Emery, C.G. Harbron, M. Wappett, A. Sharpe, M. Dymond, J.C. Barrett, E.A. Harrington, G. Marshall, Evaluating Robustness and Sensitivity of the NanoString Technologies nCounter Platform to Enable Multiplexed Gene Expression Analysis of Clinical Samples, Cancer Res 75 (2015) 2587-2593. 10.1158/0008-5472.CAN-15-0262.

[20] I.T. Joliffe, B.J. Morgan, Principal component analysis and exploratory factor analysis, Stat Methods Med Res 1 (1992) 69-95. 10.1177/096228029200100105.

[21] P. Armitage, G. Berry, Stat Meth Med Res. , Third Edition ed., Blackwell Science Ltd., Oxford, United Kingdom, 2002.

[22] J.M. Dean, I.J. Lodhi, Structural and functional roles of ether lipids, Protein Cell 9 (2018) 196-206. 10.1007/s13238-017-0423-5.

[23] K. Yamazaki, N. Masaki, Y. Kohmura-Kobayashi, C. Yaguchi, T. Hayasaka, H. Itoh, M. Setou, N. Kanayama, Decrease in Sphingomyelin (d18:1/16:0) in Stem Villi and Phosphatidylcholine (16:0/20:4) in Terminal Villi of Human Term Placentas with Pathohistological Maternal Malperfusion, PloS one 10 (2015) e0142609. 10.1371/journal.pone.0142609.

**Table 1 Summary statistics for the mothers and offspring (n = 99)**

|  |  |
| --- | --- |
|  | Summary statistics |
| Maternal age pre-pregnancy (years)1 | 28.5 (4.0) |
| Primiparous, n (%) | 45 (45%) |
| Maternal education ≥ A-levels, n (%) | 62 (63%) |
| Maternal triceps skinfold (mm)2 | 21.1 (15.9, 24.8) |
| Maternal height (m)1 | 1.62 (0.06) |
| Pre-pregnant maternal BMI (kg/m2)2 | 25.0 (23.0, 29.2) |
| Offspring gestational age at birth (weeks) 1 | 39.8 (1.3) |
| Male offspring, n (%) | 52 (53%) |
| Birthweight (kg)1 | 3.5 (0.4) |
| Placental weight (kg) 1 | 0.46 (0.08) |

1Mean (SD), 2Median (Inter-quartile range)

**Table 2, Relationships between lipid principal component scores and body composition measures in mother and offspring adjusted for infant sex and gestational age.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | principal  component 1 | | principal  component 2 | | principal  component 3 | |
|  | *β* | P | *β* | P | *β* | P |
| Woman's body mass index | -0.03 | 0.74 | -0.05 | 0.61 | -0.13 | 0.20 |
| Sum of skinfolds | -0.02 | 0.82 | -0.13 | 0.20 | -0.08 | 0.45 |
| Woman's height | 0.09 | 0.37 | 0.16 | 0.13 | -0.01 | 0.96 |
| Placental weight | 0.19 | 0.07 | 0.23 | 0.02 | 0.03 | 0.78 |
| Birthweight | 0.16 | 0.08 | 0.16 | 0.08 | 0.02 | 0.83 |
| Neonatal fat mass (DXA) | 0.11 | 0.26 | 0.05 | 0.63 | -0.01 | 0.90 |
| Neonatal lean mass (DXA) | 0.10 | 0.30 | 0.19 | 0.05 | 0.09 | 0.34 |

**Table 3, Relationships between individual placental lipid species (%) and birth parameters adjusted for infant sex and gestational age**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Placental weight** | | **Birthweight** | | **Neonatal lean mass** | | **Neonatal fat mass** | |
|  | *β* | P | *β* | P | *β* | P | *β* | P |
| **lyso.PC.a.C18.0** | 0.26 | 0.01 | 0.04 | 0.65 | 0.07 | 0.46 | 0.02 | 0.83 |
| **PC.aa.C34.1** | 0.44 | <0.001 | 0.24 | 0.008 | 0.29 | 0.002 | 0.23 | 0.01 |
| **PC.aa.C34.4** | -0.31 | 0.003 | -0.17 | 0.07 | -0.16 | 0.09 | -0.07 | 0.48 |
| **PC.aa.C36.1** | 0.29 | 0.006 | 0.30 | 0.001 | 0.32 | 0.001 | 0.24 | 0.01 |
| **PC.aa.C36.2** | 0.19 | 0.07 | 0.23 | 0.01 | 0.17 | 0.08 | 0.10 | 0.30 |
| **PC.aa.C38.5** | -0.31 | 0.003 | -0.25 | 0.005 | -0.28 | 0.002 | -0.22 | 0.02 |
| **PC.ae.C40.0** | -0.41 | <0.001 | -0.27 | 0.002 | -0.26 | 0.006 | -0.21 | 0.03 |
| **SM.a.C34.2** | 0.45 | <0.001 | 0.23 | 0.02 | 0.22 | 0.03 | 0.12 | 0.21 |
| **SM.a.C35.2** | 0.29 | 0.004 | 0.22 | 0.02 | 0.15 | 0.11 | 0.15 | 0.13 |
| **SM.a.C36.2** | 0.38 | <0.001 | 0.18 | 0.05 | 0.15 | 0.11 | 0.05 | 0.61 |

Analysis of Fisher-Yates transformed lipid variables (z-scores). Only lipid species where there was a relationship with at least one P-value ≤ 0.01 are shown. All data are shown in All data are shown in the supplementary data table S5.

**Table 4, Associations between lipid principal component scores and gene expression adjusted for infant sex and gestational age**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Principal**  **component 1** | | **principal**  **component 2** | | **principal**  **component 3** | |
| **gene** | **β** | **P** | **β** | **P** | **β** | **P** |
| *ACAT2* | -0.23 | 0.03 | 0.03 | 0.78 | 0.15 | 0.13 |
| *ALPP* | -0.05 | 0.63 | -0.19 | 0.06 | -0.10 | 0.33 |
| *ANGPTL4* | -0.08 | 0.46 | -0.08 | 0.45 | -0.12 | 0.23 |
| *APOE* | 0.28 | 0.008 | 0.33 | 0.001 | 0.02 | 0.86 |
| *CGI-58* | 0.03 | 0.76 | 0.02 | 0.82 | -0.09 | 0.37 |
| *ELOVL-1* | 0.10 | 0.35 | -0.10 | 0.35 | -0.10 | 0.30 |
| *FABP4* | -0.08 | 0.43 | -0.25 | 0.01 | -0.02 | 0.82 |
| *FADS1* | -0.17 | 0.10 | 0.17 | 0.09 | 0.04 | 0.69 |
| *FAT/CD36* | 0.13 | 0.22 | -0.06 | 0.53 | -0.02 | 0.87 |
| *FATP2* | -0.37 | <0.001 | 0.20 | 0.05 | 0.11 | 0.30 |
| *FATP3* | -0.04 | 0.74 | 0.11 | 0.30 | 0.08 | 0.42 |
| *FATP4* | -0.18 | 0.10 | 0.07 | 0.48 | 0.08 | 0.41 |
| *G0S2* | -0.21 | 0.07 | 0.30 | 0.005 | 0.03 | 0.78 |
| *GOT2* | -0.11 | 0.28 | -0.03 | 0.74 | 0.04 | 0.71 |
| *LEP* | 0.26 | 0.01 | -0.26 | 0.01 | -0.14 | 0.15 |
| *MEFD2A* | -0.00 | 0.97 | -0.19 | 0.07 | -0.12 | 0.25 |
| *PLA2G2A* | 0.00 | 0.97 | 0.18 | 0.07 | 0.16 | 0.11 |
| *PLIN2* | 0.14 | 0.20 | -0.33 | 0.001 | -0.22 | 0.03 |
| *PLIN3* | -0.15 | 0.15 | 0.03 | 0.75 | -0.25 | 0.01 |
| *PNPLA2* | -0.00 | 0.97 | -0.24 | 0.02 | -0.19 | 0.05 |
| *PPARG* | 0.10 | 0.34 | -0.09 | 0.36 | -0.02 | 0.85 |
| *SOAT1* | 0.10 | 0.33 | 0.15 | 0.14 | 0.07 | 0.49 |
| *SRB1* | 0.10 | 0.33 | -0.29 | 0.004 | -0.08 | 0.43 |
| *SREBP1* | 0.10 | 0.35 | -0.13 | 0.21 | -0.08 | 0.43 |
| *STARD3* | 0.14 | 0.20 | -0.14 | 0.18 | -0.07 | 0.51 |
| *TPSO* | -0.14 | 0.18 | 0.19 | 0.06 | 0.12 | 0.25 |

**Table 5, Associations between placental gene expression and lipid composition adjusted for infant sex and gestational age**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***APOE*** | | ***FATP2*** | | ***G0S2*** | | ***LEP*** | | ***PLIN2*** | | ***SRB1*** | | ***SREBP1*** | |
|  | **β** | **P** | **β** | **P** | **β** | **P** | **β** | **P** | **β** | **P** | **β** | **P** | **β** | **P** |
| lyso.PC.a.C16.0 | 0.02 | 0.84 | 0.40 | <0.001 | 0.25 | 0.02 | -0.04 | 0.71 | -0.16 | 0.12 | -0.07 | 0.51 | -0.18 | 0.09 |
| lyso.PC.a.C18.0 | 0.17 | 0.11 | 0.37 | <0.001 | 0.33 | 0.004 | -0.19 | 0.06 | -0.26 | 0.01 | -0.18 | 0.09 | -0.24 | 0.02 |
| lyso.PC.a.C18.1 | 0.13 | 0.21 | 0.40 | <0.001 | 0.30 | 0.007 | -0.07 | 0.50 | -0.24 | 0.02 | -0.09 | 0.40 | -0.15 | 0.15 |
| PC.aa.C32.1 | 0.11 | 0.30 | -0.33 | 0.002 | -0.29 | 0.01 | 0.34 | 0.001 | 0.20 | 0.05 | 0.21 | 0.04 | 0.07 | 0.54 |
| PC.aa.C32.2 | 0.11 | 0.32 | -0.43 | <0.001 | -0.24 | 0.04 | 0.24 | 0.02 | 0.10 | 0.33 | 0.10 | 0.33 | 0.08 | 0.48 |
| PC.aa.C34.4 | -0.07 | 0.47 | -0.37 | <0.001 | -0.11 | 0.32 | 0.31 | 0.002 | 0.31 | 0.002 | 0.26 | 0.008 | 0.22 | 0.03 |
| PC.aa.C36.1 | 0.34 | 0.001 | -0.19 | 0.07 | -0.00 | 0.99 | 0.14 | 0.17 | 0.00 | 0.97 | 0.02 | 0.88 | -0.00 | 0.99 |
| PC.aa.C36.3 | -0.03 | 0.76 | -0.17 | 0.11 | -0.33 | 0.004 | 0.23 | 0.03 | 0.20 | 0.06 | 0.22 | 0.03 | 0.37 | <0.001 |
| PC.aa.C36.5 | -0.24 | 0.02 | -0.17 | 0.10 | -0.16 | 0.15 | 0.29 | 0.004 | 0.40 | <0.001 | 0.23 | 0.03 | 0.15 | 0.16 |
| PC.aa.C38.6 | -0.20 | 0.05 | 0.36 | <0.001 | 0.23 | 0.04 | -0.36 | <0.001 | -0.19 | 0.07 | -0.13 | 0.21 | -0.13 | 0.20 |
| PC.aa.C40.4 | 0.35 | 0.001 | -0.08 | 0.46 | 0.22 | 0.05 | -0.08 | 0.46 | -0.11 | 0.29 | -0.16 | 0.13 | -0.19 | 0.07 |
| PC.ae.C34.2 | 0.14 | 0.16 | 0.15 | 0.14 | 0.00 | 0.98 | -0.21 | 0.04 | -0.32 | 0.001 | -0.21 | 0.03 | -0.08 | 0.45 |
| PC.ae.C34.3 | 0.13 | 0.21 | 0.16 | 0.13 | 0.09 | 0.44 | -0.19 | 0.07 | -0.27 | 0.007 | -0.32 | 0.001 | -0.16 | 0.12 |
| PC.ae.C36.1 | 0.33 | 0.001 | -0.16 | 0.13 | 0.02 | 0.83 | 0.03 | 0.78 | -0.09 | 0.37 | 0.00 | 0.96 | 0.07 | 0.50 |
| PC.ae.C38.5 | -0.04 | 0.71 | 0.35 | <0.001 | 0.31 | 0.004 | -0.34 | 0.001 | -0.30 | 0.002 | -0.23 | 0.02 | -0.15 | 0.13 |
| PC.ae.C38.6 | 0.07 | 0.51 | 0.43 | <0.001 | 0.34 | 0.002 | -0.38 | <0.001 | -0.33 | 0.001 | -0.33 | 0.001 | -0.22 | 0.03 |
| PC.ae.C40.5 | 0.28 | 0.005 | 0.11 | 0.26 | 0.38 | <0.001 | -0.26 | 0.008 | -0.27 | 0.006 | -0.25 | 0.01 | -0.11 | 0.28 |
| PC.ae.C40.6 | 0.20 | 0.05 | 0.30 | 0.003 | 0.40 | <0.001 | -0.28 | 0.005 | -0.30 | 0.002 | -0.22 | 0.03 | -0.14 | 0.18 |
| SM.a.C34.2 | 0.22 | 0.04 | 0.18 | 0.09 | 0.35 | 0.001 | -0.24 | 0.02 | -0.32 | 0.001 | -0.30 | 0.003 | -0.41 | <0.001 |
| SM.a.C35.1 | 0.36 | 0.001 | -0.18 | 0.08 | 0.01 | 0.96 | 0.09 | 0.38 | 0.07 | 0.53 | 0.09 | 0.42 | 0.10 | 0.34 |
| SM.a.C36.1 | 0.25 | 0.02 | -0.34 | 0.001 | -0.15 | 0.19 | 0.27 | 0.009 | 0.28 | 0.007 | 0.29 | 0.005 | 0.25 | 0.02 |
| SM.a.C36.2 | 0.37 | <0.001 | -0.10 | 0.37 | 0.18 | 0.12 | 0.06 | 0.56 | 0.02 | 0.83 | -0.02 | 0.88 | -0.14 | 0.19 |
| SM.a.C42.6 | -0.20 | 0.05 | 0.29 | 0.004 | 0.21 | 0.06 | -0.35 | <0.001 | -0.19 | 0.06 | -0.10 | 0.34 | -0.12 | 0.23 |

Genes are listed in the table where there were at least two lipid associations at P ≤ 0.001. All data are shown in the supplementary data table S6. Data is adjusted for sex and gestational age.

**Figure 1. Placental polar lipid concentration (µmol/kg).**