**Electronic Supplementary Material**

**Sphingolipids and acylcarnitines are altered in placentas from women with gestational diabetes mellitus**

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Short version of the title: placental lipids in gestational diabetes

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**Supplementary File S1.: Assessment of Dietary intake**

S1. Assessment of Dietary Intake

Two 24-h recall (24hR) were collected in the 3rd trimester of pregnancy: one face-to-face and another up to 2 days later, by a phone interview. This approach was already validated by Barbieri et al. (2016) (1) to access the dietary intake of Brazilian pregnant people. Trained nutritionists systematized the portions and home measures, as well as the insertion of dietary data into the database. Nutrient intake was analyzed by the software DietBox® v.4.0, Rio de Janeiro, Brazil, using the Tabela Brasileira de Composição de Alimentos (2) and Instituto Brasileiro de Geografia e Estatística (3) database. Habitual nutrient intake was estimated by the Multiple Source Method, a statistical method that considers intra- and inter-individual variation over the same period using at least two different dietary inputs (e.g., dietary recalls and/or food frequency questionnaire (4). It has been shown to be adequate to estimate usual intake during pregnancy (5).

**Supplementary File S2.: *Lipid profile analysis of placental samples***

 S*2.1 Extraction of placental lipids*

Lipids were extracted using a modified Folch method (6) and one technical replicate was performed for each sample. Briefly, 0.35 mL of cold methanol and 0.35 mL of chloroform were added to vials containing ~ 50 mg of frozen placental tissue and one metallic bead and were disrupted in a single batch (Tissue Lyser II, Qiagen; 2 ×2.5 min, 17 s−1, Hilden, Germany). Next, following addition of 0.35 mL of chloroform and 0.15 mL of ultrapure water (Chromasolv; Honeywell, Reidel-de Haën, Thermo Fisher Scientific, Waltham, MA, USA), samples were thoroughly vortexed for 30 seconds and centrifuged (17,000 × *g*, 10 min). The lower phase containing the lipid extract was transferred to glass vials, dried under a gentle stream of nitrogen and stored at −80 °C until further analysis.

Quality control (QC) samples consisted of a pooled sample containing 10 μL aliquots of each sample. QC samples were analysed in 3 technical replicates.

*S.2.2. Ion Mobility QTOF LC/MS Lipid Profile Analysis of Placental Samples*

An Agilent 6560 Ion mobility Quadrupole Time-of-Flight (DTIMQTOF) LC-MS system coupled with an Agilent 1290 UHPLC system was used to combine separation power and selectivity of Liquid Chromatography (LC), Drift Time Ion Mobility (DTIM), and Mass spectrometry (MS) techniques. The Dual Agilent Jet Stream electrospray ionization source was operated separately in positive and negative ion mode.

The dried chloroform phase was suspended in 50 μL methanol containing the internal standards (24 deuterated lipids, representatives of phosphatidic acid, phosphatidylcholines, phosphatidylethanolamines, glycerophospholipids, phosphatidylinositols, phosphatidylserine, sphingomyelins, ceramides, triacylglycerols and fatty acids (Supplementary Table S1) and 75 μL of a solution of isopropanol:acetonitrile:water (IPA:ACN:H2O, 2:1:1, v/v). All samples were analysed in positive and negative mode. For positive mode, the samples and QC were diluted 1:10 with the IPA:ACN:H2O (2:1:1) solution.

The lipid extract was separated in a ACQUITY CSH C18 column (100 × 2.1 mm and 1.7 μm particle diameter, Waters) conditioned at 55 °C. The mobile phase consisted of: (A) 10 mM ammonium formate solution in 40 % ultrapure water and 60 % acetonitrile and (B) 10 mM ammonium formate solution in 90 % isopropanol, and 10 % acetonitrile (v/v). The mobile phase was pumped at a flow rate of 400 μL/min starting at 60% of A and 40% of B. Solvent B was increased linearly to 43% in 2 min, to 50% at 2.1 min, to 54% at 12 min and then to 99% in 18 min. At 18.1 min solvent B was brought back to the initial conditions and remained at this percentage for 1.9 min. The column was re-equilibrated for 3 min at 40% solvent B. An Agilent tuning solution was injected before the analysis to tune the instrument in the m/z range 100–1700, and before every 10 samples to perform CCS re-calibration. During samples acquisition, an Agilent reference mix was constantly injected for mass re-calibration. The Agilent Mass Hunter LC-MS acquisition console was used for data acquisition.

*S2.2.1 Positive Ion Mode*

The electrospray capillary potential was set to 60 V, the needle at 20 kV. Nitrogen gas at 5 L/min (set at 48 mTorr) and 375 °C was used as drying gas for solvent evaporation; sheath gas was set at 275 °C and 12 L/min flow rate. Full-scan spectra were obtained in the ranges of 50– 1200 amu, scan time of 0.20 sec., scan width of 0.70 amu, and detector set at 2950 V.

*S2.2.2 Negative Ion Mode*

The electrospray capillary potential was set to 60 V, and the needle at 20 kV. Nitrogen gas at 5 L/min (set at 48 mTorr) and at 375 °C was used as drying gas for solvent evaporation, sheath gas was set at 275 °C and 12 L/min flow rate. Full-scan spectra were obtained in the ranges of 50–1200 amu, 0.20 sec can time, 0.70 amu scan width, and detector set at 2950 V.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Chemical notation** | **Class** | **Manufactor** |
| N-palmitoyl-d31-D-erythro-sphingosine (C16-d31 Ceramide) | C16-d31 Ceramide | Cer | Avanti |
| 1-palmitoyl-d31-2-oleoyl-sn-glycero-3-phosphate (sodium salt) (16:0-d31-18:1 PA) | 16:0-d31-18:1 PA | PA | Avanti |
| 1-palmitoyl(D31)-2-oleyl-sn-glycero-3-phosphocholine (16:0-d31-18:1 PC) | 16:0-d31-18:1 PC | PC | Avanti |
| 1-palmitoyl(D31)-2-oleyl-sn-glycero-3-phosphoethanolamine (16:0-d31-18:1 PE) | 16:0-d31-18:1 PE | PE | Avanti |
| 1-palmitoyl-d31-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (16:0-d31-18:1 PG) | 16:0-d31-18:1 PG | PG | Avanti |
| 1-palmitoyl-d31-2-oleoyl-sn-glycero-3-phosphoinositol (ammonium salt) (16:0-d31-18:1 PI) | 16:0-d31-18:1 PI | PI | Avanti |
| 1,2-dimyristoyl-d54-sn-glycero-3[phospho-L-serine] (sodium salt)(14:0 PS-d54) | 14:0 PS-d54 | PS | Avanti |
| N-palmitoyl(D31)-d-erythro-sphingosylphosphorylcholine (16:0-d31 SM) | 16:0-d31 SM | SM | Avanti |
| 1,2-dipalmitoyl-d62-sn-glycero-3-[phospho-L-serine] (sodium salt) | 16:0 PS-d62 | PS | Qmx |
| cholesteryl-2,2,3,4,4,6-d6-octadecanoate (18:0-d6 CE) | 18:0-d6 CE | Cer | Qmx |
| Pentadecanoic-d29 Acid (15:0-d29 FA) | 15:0-d29 FA | FA | Qmx |
| Heptadecanoic-d33-acid (17:0-d33 FA) | 17:0-d33 FA | FA | Qmx |
| Eicosanoic-d39 Acid (20:0-d39 FA) | 20:0-d39 FA | FA | Qmx |
| Tetradecylphosphocholine-d42 (14:0-d29 LPC-d13) | 14:0-d29 LPC-d13 | LysoPC | Qmx |
| Glyceryl Tri(pentadecanoate-d29) (45:0-d29 TG) | 45:0-d29 TG | TAG | Qmx |
| Glyceryl-tri(hexadecanoate-d31) (48:0-d31 TG) | 48:0-d31 TG | TAG | Qmx |
| Glyceryl Tri(octadecanoate-d35) (54:0-d35 TG) | 54:0-d35 TG | TAG | Cayman Chemicals |
| Arachidonic acid d8 | arachidonic acid d8 | PUFA | Cayman Chemicals |
| Llinoleic acid d4 | linoleic acid d4 | PUFA | Cayman Chemicals |
| 11(12)-EET-d11 | 11(12)-EET-d11 | oxFA | Cayman Chemicals |
| Prostaglandin E2-d4 | Prostaglandin E2-d4 | oxFA | Cayman Chemicals |
| 12-HETE-d8 | 12-HETE-d8 | oxFA | Cayman Chemicals |
| TxB2-d4 | TxB2-d4 | oxFA | Cayman Chemicals |
| LTB2-d4 | LTB2-d4 | oxFA | Cayman Chemicals |

***Supplementary Table S1.*** *Internal standards used in the high-resolution ion-mobility lipidomics.*

Cer:Ceramides; PA: Phosphatidic Acids; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Phosphatidylglycerol; PI: Phosphatidylinositol; PS: Phosphatidylserine; SM: Sphingomyelin; FA:Fatty acyl; LysoPC:Lysophosphatidilcholine; TAG: Triacylglycerol; PUFA:Poliunsaturated fatty acid; oxFA:oxidated fatty acid

***Supplementary Table S2****.*

*Energy and nutrient intake of women participating in the study.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nutrient** | **Intake a** | | **Inadequacy**  **(Prevalence %) b** | | | | **Reference Value**  **(Daily intake) c** |
| **nGDM** | **GDM** | **nGDM** | **GDM** | |  | |
| Energy (En) (Kcal) | 2104 (1820 - 2313) | 1968 (1794 - 2201) | - | - | | - | |
| Protein (%En) | 16.85 (15.42 - 21.40) | 16.79 (15.69 -17.77) | 0 | 0 | | 10-35% | |
| Carbohydrate (%En) | 53.11 (50.99 - 57.01) | 54.44 (53.44 -55.35) | 0 | 0 | | 45-65% | |
| Lipids (%En) | 31.18 (27.55 - 31.65) | 29.94 (29.30 -31.78) | 0 | 0 | | 20-35% | |
| Cholesterol (mg) | 285.50 (285.10 - 285.60) | 285.30 (284.60 - 286.20) | - | - | | - | |
| Total sugar (%En) | 3.91 (3.48 – 4.29) | 4.07 (3.58 – 4.53) | 0 | 0 | | <10% | |
| Fiber (g) | 20.14 (15.75 - 26.12) | 23.42 (20.82 -33.83) | 85.71 | 66.67 | 28 | | |
| Sodium (mg) | 2370 (1805 - 2596) | 2227 (1726 - 2746) | 42.86 | 50 | | 2.300 | |
| Ultra processed food (%En) | 31.85 (25.3 - 45.19) | 24.02 (18.55 -31.88) | - | - | | - | |
| *Fatty acids* |  |  |  |  | |  | |
| Polyunsaturated (%En) | 5.14 (4.24 - 6.19) | 5.06 (4.24 - 5.25) | 42.86 | 50 | | 5-10% | |
| Monounsaturated (%En) | 9.14 (8.71 - 9.60) | 9.63 (8.63 - 10.57) | 85.71 | 66.67 | | 10-15% | |
| Saturated (%En) | 10.65 (10.42 - 10.81) | 10.88 (10.34 - 11.1) | 100 | 100 | | <10% | |
| Trans fatty acid (%En) | 1.13 (0.89 - 1.50) | 1.24 (1.00 - 1.46) | - | - | | - | |

nGDM: Women with healthy pregnancies (see methods for eligibility criteria); GDM: Women with gestational diabetes mellitus. 24hR-MSM: four 24-hour recalls were used and data was assessed by the *Multiple Source Method.*

aNutrients are expressed as median and interquartile range (p25-75) and compared by Mann-Whitney test. bInadequacy based on Estimated Average Requirement (EAR) (IOM, 2006). cDRI: Dietary Reference Intakes for pregnant women (IOM, 2006): macronutrient intake expressed as Acceptable Macronutrient Distribution Range (AMDR) and fiber intake expressed as Recommended Dietary Allowance (RDA);

***Supplementary Table S3.*** *Lipid categories and classes identified in placental samples according to the high-resolution ion-mobility lipidomics.*

|  |  |
| --- | --- |
| ***Lipid category***  ***(% annotated features)*** | **Number of lipid species assigned in each category** |
| *Glycerophospholipids 53.74 %* | 121 Phosphatidylcholine |
| 42 Phosphatidylinositol |
| 40 Phosphatidylglycerol |
| 36 Phosphatidylserine |
| 25 Cardiolipin |
| 24 Phosphatidic acid |
| 22 Glycerophosphate |
| 20 Phosphatidylethanolamine |
| 5 LysoPC |
| 2 LysoPE |
| *Glycerolipids 21.21%* | 97 Triacylglycerol |
| 30 Diacylglycerol |
| 2 Monoacylglycerol |
| 3 Sulfoquinovosyldiacylglycerol |
| 1 Monogalatosyldiacyglycerol |
| *Sphingolipids. 20.25 %* | 56 Ceramide |
| 30 Sphingomyelin |
| 16 Glyceroceramide |
| 11 Lactosylceramide |
| 6 Ceramide phosphoethanolamine |
| 6 Ceramide phosphoinositol |
| 2 Ganglioside |
| *Fatty acyls 3.19 %* | 13 Fatty acid |
| 7 Acylcarnitine |
| *Sterols 1.59 %* | 8 Glycerocholesterol |
| 2 Cholesteryl ester |

Categories values were expressed relative to total lipids identified in placentas by UPLC-DTIMS-QTOF-MS.

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***Supplementary figure S1****: Flow diagram of participants’ recruitment for the cohort study and final sample size.*

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***Supplementary figure S2****: Relative abundance of lipid categories identified by high-resolution ion-mobility lipidomics*. nGDM: placentas from healthy pregnancies (see methods for eligibility criteria); GDM: placentas from pregnancies with gestational diabetes mellitus. The relative abundance was calculated considering all annotated features; Data represented by median with interquartile range; Comparative analysis between groups by Mann-Whitney test.

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***Supplementary figure S3****: Relative abundance of lipid categories identified in the placenta by high-resolution ion-mobility lipidomics according to mode of delivery vaginal (n=4) or caesarean section (n=3) in the nGDM group*.

***Supplementary Table S4:*** *Lipid species with Variable Importance in the Projection (VIP) scores ≥ 0.95 and loading factors (PLS-regression coefficient) for Principal component 1 according to the PLS-DA multivariate. Lipid species are grouped according to lipid categories.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **VIP Scorea**  **( ≥ 0.95)** | **Loading Factorb** | **% CVc** |
| **Glycerophospholipids (GP)*d*** |  |  |  |
| ***Phosphatidic acid (PA)e*** |  |  |  |
| PA 37:1 | 2.4031 | -0.11523 | 11.47 |
| PA 44:8 | 1.9298 | -0.041713 | 5.84 |
| PA 42:6 | 1.6674 | -0.0063421 | 16.74 |
| PA 42:4 | 1.4573 | -0.067196 | 7.39 |
| PA 30:1  PA 40:5 | 1.4532  1.0143 | -0.086594  -0.049591 | 13.83  12.82 |
| ***Phosphatidylcholine (PC) e*** |  |  |  |
| PC 33:3 | 3.7288 | 0.11032 | 18.38 |
| PC 40:4 | 2.8833 | -0.05145 | 9.61 |
| PC 35:6 | 2.6057 | 0.006273 | 13.90 |
| PC 37:1 | 2.3286 | -0.095168 | 13.08 |
| PC 36:4 | 2.2889 | 0.003807 | 15.33 |
| PC 34:1 | 2.1586 | -0.02564 | 19.82 |
| PC 40:1 | 2.1377 | -0.045006 | 2.37 |
| PC 35:2 | 2.0465 | 0.038999 | 17.11 |
| PC 42:11 | 1.7886 | -0.065869 | 13.96 |
| PC 31:0 | 1.6897 | 0.051061 | 16.16 |
| PC 29:0 | 1.6492 | -0.00064806 | 18.69 |
| PC 32:0 | 1.6098 | -0.063271 | 17.90 |
| PC 36:4 | 1.5748 | -0.063066 | 9.37 |
| PC 37:6 | 1.1857 | 0.053032 | 18.17 |
| PC 33:1 | 1.1836 | 0.031205 | 3.60 |
| PC 37:4 | 1.0856 | 0.033167 | 13.75 |
| PC 44:5  PC 42:2  PC 38:6  PC 44:5  PC 37:7 | 1.0571  1.0561  1.0443  1.0227  1.0007 | -0.13156  0.036102  -0.023235  -0.083132  -0.015018 | 14.60  14.12  16.23  11.23  8.91 |
| ***Phosphatidylethanolamine (PE) e*** |  |  |  |  |  |  |
| PE 38:8 | 1.341 | -0.065271 | 9.52 |
| PE 36:5 | 1.2779 | -0.056364 | 12.27 |
| PE 40:6 | 1.153 | 0.021736 | 9.11 |
| ***Phosphatidylglycerol (PG) e*** |  |  |  |
| PG 36:4 | 1.7352 | -0.007282 | 8.26 |
| PG 33:5 | 1.5585 | -0.085686 | 7.57 |
| PG 44:10 | 1.3474 | 0.027155 | 12.38 |
| PG 42:4 | 1.1774 | -0.054186 | 17.53 |
| PG 38:4 | 1.1165 | -0.052907 | 19.61 |
| PG 34:3 | 1.0205 | -0.01403 | 15.99 |
| PG 38:3 | 0.98901 | -0.063089 | 16.72 |
| ***Phosphatidylinositol (PI) e*** |  |  |  |
| PI 34:1 | 4.9208 | 0.075769 | 13.55 |
| PI 39:1 | 1.5521 | -0.1233 | 11.62 |
| PI 40:7 | 1.1561 | -0.052887 | 3.78 |
| PI 38:4 | 1.1255 | -0.038335 | 16.65 |
| PI 42:4  PI 38:5  PI 37:4 | 1.1002  1.0571  0.98984 | -0.034496  -0.033094  -0.056184 | 15.73  13.70  12.25 |
| ***Phosphatidylserine (PS) e*** |  |  |  |
| PS 37:1 | 3.3838 | -0.19559 | 11.44 |
| PS 44:6 | 2.399 | -0.040199 | 8.43 |
| PS 42:6 | 2.1523 | -0.16394 | 17.82 |
| PS 38:4 | 1.8224 | -0.055739 | 16.15 |
| PS 39:6 | 1.3422 | -0.059073 | 15.26 |
| PS 37:2 | 1.3034 | 0.038166 | 18.82 |
| PS 34:2  PS 44:7 | 1.1558  0.99236 | -0.085521  -0.022496 | 18.79  6.71 |
| ***Cardiolipin (CL) e*** |  |  |  |
| CL (1'-[18:0/18:1].3'-[20:0/20:0])  CL(1'-[16:0/18:1].3'-[18:2/20:4] | 2.0691  0.98704 | -0.059862  -0.12658 | 3.03  18.53 |
| ***Sphingolipids d*** |  |  |  |
| ***Ceramides e*** |  |  |  |
| Cer 34:2 | 3.4983 | -0.10626 | 18.41 |
| Cer 34:0 | 3.4768 | -0.085095 | 13.12 |
| Cer 42:2 | 3.3919 | -0.068881 | 8.96 |
| Cer 36:0 | 3.3855 | -0.017291 | 17.75 |
| Cer 42:0 | 3.1461 | -0.091087 | 13.10 |
| Cer 32:1 | 2.9289 | -0.072722 | 8.55 |
| Cer 34:1  Cer 32:1 | 2.8807  2.3345 | -0.080431  -0.056876 | 5.31  7.00 |
| Cer 44:0 | 2.0705 | -0.0283 | 8.18 |
| Cer 34:0 | 1.7491 | -0.053497 | 7.99 |
| Cer 35:1 | 1.6462 | -0.050531 | 10.81 |
| Cer 40:1 | 1.6452 | -0.11413 | 9.96 |
| Cer 34:2 | 1.6407 | -0.053974 | 10.77 |
| Cer 34:0 | 1.585 | -0.049373 | 3.40 |
| Cer 44:2 | 1.5213 | -0.044076 | 15.30 |
| Cer 36:1 | 1.4612 | -0.054402 | 10.63 |
| Cer 41:1 | 1.3968 | -0.041737 | 14.99 |
| Cer 43:1 | 1.3859 | -0.087955 | 10.65 |
| Cer 34:2 | 1.2581 | -0.033761 | 5.03 |
| Cer 44:1 | 1.2548 | -0.033752 | 14.51 |
| Cer 42:2  Cer 38:0  Cer 44:2  Cer 34:2  Cer 42:2 | 1.0322  1.021  0.98208  0.97005  0.96162 | -0.014624  -0.03612  -0.034135  -0.027701  -0.012192 | 7.26  15.29  10.12  10.77  14.42 |
| ***Phosphatidylethanolamine-Ceramide (PE-Cer) e*** | | |  |
| PE-Cer 32:2 | 1.9242 | -0.10563 | 9.35 |
| PE-Cer 38:3 | 1.7463 | -0.069984 | 19.77 |
| ***Phosphatidylinositol-Ceramide (PI-Cer) e*** | | |  |
| PI-Cer 38:0 | 1.6119 | -0.1187 | 15.96 |
| ***Sphingomyelins (SM) e*** | | |  |
| SM 42:1  SM 36:1  SM 40:0 | 1.2792  1.0091  0.98896 | -0.023837  0.021301  -0.12658 | 19.84  18.50  10.09 |
| ***Glucosylceramides (Glc-Cer) e*** | | |  |
| GlcCer 34:1  GlcCer 44:1 | 2.0818  1.7574 | 0.053149  -0.05635 | 7.74  11.08 |
| GlcCer 38:3 | 1.2565 | -0.053364 | 15.89 |
| GlcCer 40:1 | 0.96909 | -0.1292 | 18.61 |
| ***Lactosylceramides (Lac-Cer) e*** | | |  |
| LacCer 34:1 | 1.9529 | -0.00074677 | 6.08 |
| LacCer 32:1 | 1.948 | -0.041391 | 11.97 |
| ***Gangliosids e*** |  |  |  |
| KDNalpha2-3Galbeta1-4Glcbeta-Cer(d18:1/16:0) | 0.97173 | -0.12185 | 18.88 |
| ***Free Fatty Acid**d*** |  |  |  |
| Stearic Acid | 2.0225 | 0.078015 | 9.18 |
| ***Acylcarnitinesd*** |  |  |  |
| 9,12-Hexadecadienylcarnitine | 1.7427 | 0.0013303 | 17.45 |
| Acetyl-D-carnitine | 1.2939 | 0.046074 | 12.97 |
| Acylcarnitine C18:0 | 1.1018 | 0.0059887 | 17.40 |
| O-(17-carboxyheptadecanoyl)carnitine | 1.0295 | -0.031774 | 17.03 |
| ***Glycerolipids (GL)d*** | | |  |
| ***Sulfoquinovosyl diacylglycerol e*** | | |  |
| SQDG 38:6 | 3.5373 | 0.15981 | 15.76 |
| SQDG 38:6 | 1.1462 | 0.028656 | 19.05 |
| ***Monogalactosyldiacylglycerol e*** |  |  |  |
| MGDG 36:8 | 1.0443 | -0.0087515 | 16.23 |
| ***Diacylglycerol (DG) e*** |  |  |  |
| DG 42:9 | 4.8509 | -0.19683 | 17.49 |
| DG 36:1 | 2.9179 | -0.13883 | 15.93 |
| DG 38:4 | 2.5185 | 0.027592 | 15.76 |
| DG 36:8 | 1.8704 | 0.046204 | 15.83 |
| DG 44:1 | 1.826 | 0.077649 | 18.79 |
| DG 28:2 | 1.2474 | -0.058845 | 19.16 |
| DG 33:3  DG 37:2 | 1.0704  1.0052 | -0.032621  0.00020279 | 18.62  8.53 |
| ***Triacylglycerol (TG) e*** | | |  |
| TG 51:7 | 2.9034 | -0.21341 | 4.27 |
| TG 58:9 | 2.452 | -0.028145 | 12.59 |
| TG 52:1 | 2.2945 | -0.062006 | 15.59 |
| TG 50:2 | 2.2107 | -0.052703 | 6.10 |
| TG 54:9 | 2.0823 | -0.049684 | 4.40 |
| TG 60:9 | 1.9573 | -0.15944 | 7.73 |
| TG 58:9 | 1.9372 | -0.053412 | 14.26 |
| TG 58:5 | 1.7533 | -0.047849 | 9.07 |
| TG 58:2 | 1.6982 | -0.038097 | 18.02 |
| TG 56:12 | 1.5397 | -0.14704 | 11.32 |
| TG 51:1 | 1.475 | -0.059098 | 5.23 |
| TG 56:9 | 1.1722 | -0.027086 | 7.62 |
| TG 57:1  TG 56:10  TG 56:12  TG 54:9  TG 51:4  TG 51:2  TG 56:11  TG 51:1  TG 51:1  TG 64:16  TG 52:4  TG 58:13 | 1.1156  1.082  1.077  1.0591  1.0391  1.0338  1.0108  1.0073  1.0017  0.99793  0.96485  0.96378 | -0.018178  -0.14704  -0.12849  0.000079  -0.12981  -0.022552  -0.13866  -0.015387  -0.02995  -0.022496  0.024472  -0.12693 | 17.24  16.98  1.28  17.90  14.56  16.15  7.56  15.47  15.45  17.68  11.91  14.26 |
| ***Sterol lipidsd*** | | |  |
| 22:2-Glc-Sitosterol | 5.8588 | -0.22858 | 19.08 |
| 22:2-Glc-Sitosterol | 1.7461 | -0.06619 | 16.60 |

PA: Phosphatidic Acid; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Phosphatidylglycerol; PI: Phosphatidylinositol; PS: Phosphatidylserine; CL: Cardiolipin.

Cer:Ceramide; PE-Cer: Phosphatidylethanolamine-Ceramide; PI-Cer: Phosphatidylinositol-Ceramide; SM: Sphingomyelin; GlcCer: Glucosylceramide; LacCer: Lactosylceramide; DG: Diacylglycerol; TG: Triacylglycerol.

**a**VIP-scores and **b**loading factors for each lipid variable on component 1 according to PLS-DA; ***c*** CV %: Coefficient of variation in the quality control (QC) samples for the respective annotated lipid specie; ***d***Lipid categories are displayed according to the frequency of features in the VIP ≥ 0.95 (higher to lower); eLipid classes were expressed as the number of carbons: number of double bonds.

***Supplementary figure S4:*** *Phospholipids with odd-chain fatty acids with discriminant power according to the PLS-DA multivariate statistics*. **(A)** Sum of 37:1 species of phosphatidylserine (PS), phosphatidic acid (PA) and phosphatidylcholine (PC). **(B)** Sum of total of PC+PA+PS 37:1 species; **(C)** PC/PA, PS/PA and PC/PS 37:1 ratios. nGDM: placentas from healthy pregnancies (see methods for eligibility criteria); GDM: placentas from gestational diabetes mellitus pregnancies. Data represented as average + standard deviation; No significant differences were observed between groups according to unpaired Student’s t test.

**Supplementary Table S5.** Placentalfatty-acylcarnitines identified by MS/MS analysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Acylcarnitines | tR  (min) | Precursor ion (m/z) | Product ion (m/z) | CE(V) | RF Lens (V) | |
| C0 | 0.89 | 162.1 | 85.1 | 23 | 71 |
| C3 | 3.25 | 218.1 | 85.0 | 20 | 78 |
| C4 | 4.01 | 232.1 | 85.2 | 23 | 78 |
| C4-DC | 6 | 318.2 | 85.166 | 23 | 94 |
| C5:1 | 4.62 | 244.1 | 85.2 | 21 | 81 |
| C5 | 4.94 | 246.2 | 85.2 | 21 | 81 |
| C6 | 5.88 | 260.2 | 85.2 | 21 | 81 |
| C8:1 | 6.5 | 286.2 | 85.2 | 23 | 94 |
| C8 | 7.37 | 288.2 | 85.2 | 23 | 94 |
| C10:2 | 7.5 | 312.2 | 85.2 | 23 | 94 |
| C10 | 8.17 | 316.2 | 85.2 | 23 | 94 |
| C5-DC | 7.46 | 332.2 | 85.2 | 23 | 94 |
| C12:1 | 8.4 | 342.2 | 85.1 | 26 | 122 |
| C12 | 9.01 | 344.2 | 85.1 | 26 | 122 |
| C14-OH | 9.04 | 388.3 | 85.1 | 26 | 122 |
| C14:2 | 8.17 | 368.2 | 85.1 | 26 | 122 |
| C14:1 | 9.2 | 370.3 | 85.1 | 26 | 122 |
| C14 | 9.46 | 372.3 | 85.1 | 26 | 122 |
| C8-DC | 9.46 | 374.3 | 85.1 | 26 | 122 |
| C15 | 9.64 | 386.3 | 85.1 | 27 | 123 |
| C16:1-OH | 9.01 | 414.3 | 85.1 | 27 | 123 |
| C16:2 | 9.35 | 396.3 | 85.1 | 27 | 123 |
| C16:1 | 9.55 | 398.3 | 85.1 | 27 | 123 |
| C16-OH | 9.51 | 416.3 | 85.1 | 27 | 123 |
| C16 | 9.77 | 400.3 | 85.1 | 27 | 123 |
| C17 | 9.61 | 414.3 | 85.1 | 27 | 123 |
| C18:2-OH | 9.36 | 440.3 | 85.1 | 27 | 123 |
| C18:1-OH | 9.59 | 442.3 | 85.1 | 27 | 123 |
| C18-OH | 9.82 | 444.3 | 85.1 | 27 | 123 |
| C18:2 | 9.66 | 424.3 | 85.1 | 27 | 123 |
| C18:1 | 9.81 | 426.3 | 85.1 | 27 | 123 |
| C18 | 9.98 | 428.3 | 85.1 | 27 | 123 |
| C20:4 | 9.63 | 448.3 | 85.1 | 27 | 123 |
| C20:2 | 9.88 | 452.3 | 85.1 | 27 | 123 |
| C20:1 | 10 | 454.3 | 85.1 | 27 | 123 |
| C20 | 10 | 456.3 | 85.1 | 27 | 123 |

***Supplementary table S6.*** *Comparison**of placental**fatty-acylcarnitines identified by the MS/MS analysis between nGDM and GDM groups.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Acylcarnitines** | **nGDM** | **GDM** | ***p* value** |
| **Short-chain fatty-acylcarnitines a** | | |  |
| C3 | 0.080 (0.048 – 0.109) | 0.163 (0.079 – 0.242) | 0.051 |
| C4 | **0.086 (0.083 – 0.011)** | **0.030 (0.019 – 0.041)** | **0.001** |
| C4DC | 2E-05 (0.000 – 0.000) | 3E-05 (.000 – 0.000) | 0.601 |
| C5 | 0.038 (0.027 – 0.085) | 0.021 (0.012 – 0.178) | 0.295 |
| C5-OH | 0.004 (0.003 – 0.007) | 0.003 (0.003 – 0.003) | 0.366 |
| C5:1 | 0.003 (0.002 – 0.007) | 0.001 (0.001 – 0.002) | 0.051 |
| C5DC | **0.003 (0.002 – 0.003)** | **0.001 (0.001 – 0.002)** | **0.035** |
| C6 | 0.057 (0.047 – 0.078) | 0.040 (0.021 – 0.076) | 0.295 |
| **Medium-chain fatty-acylcarnitines b** | | |  |
| C8 | 0.006 (0.005 – 0.008) | 0.002 (0.001 - 0.006) | 0.101 |
| C8:1 | 0.001 (0.000 – 0.001) | 0.001 (0.001 – 0.002) | 0.101 |
| C10 | **0.006 (0.004 – 0.008)** | **0.002 (0.001- 0.004)** | **0.022** |
| C10:2 | 0.001 (0.000 – 0.001) | 0.000 (0.000 – 0.000) | 0.366 |
| C12 | **0.008 (0.005 – 0.010)** | **0.001 (0.001 - 0.004)** | **0.014** |
| C12:1 | 0.001 (0.001 – 0.002) | 0.0004 (0.000 – 0.001) | 0.051 |
| **Long-chain fatty-acylcarnitines c** | | |  |
| C14 | **0.050 (0.031 – 0.062)** | **0.007 (0.005 – 0.024)** | **0.014** |
| C14-OH | **0.006 (0.003 – 0.006)** | **0.008 (0.000 – 0.002)** | **0.005** |
| C14:1 | 0.013 (0.002 – 0.014) | 0.002 (0.001 – 0.008) | 0.051 |
| C14:2 | 0.007 (0.003 – 0.008) | 0.002 (0.001 – 0.003) | 0.051 |
| C15 | **0.004 (0.003 – 0.005)** | **0.001 (0.001 – 0.003)** | **0.014** |
| C16 | **0.098 (0.080 – 0.128)** | **0.023 (0.011 – 0.081)** | **0.008** |
| C16-OH | **0.011 (0.008 – 0.014)** | **0.001 (0.000 – 0.002)** | **0.002** |
| C16:1 | **0.014 (0.013 – 0.016)** | **0.002 (0.001 – 0.007)** | **0.022** |
| C16:1-OH | **0.004 (0.003 – 0.009)** | **0.001(0.001 – 0.002)** | **0.014** |
| C16:2 | 0.001 (0.005 – 0.001) | 0.001 (0.001 – 0.001) | 0.366 |
| C17 | **0.004 (0.003 – 0.008)** | **0.001(0.000 – 0.002)** | **0.008** |
| C18 | **0.051 (0.042 – 0.113)** | **0.012 (0.005 – 0.019)** | **0.014** |
| C18:OH | **0.006 (0.003 – 0.008)** | **0.000 (0.000 – 0.001)** | **0.002** |
| C18:1 | **0.060 (0.046 – 0.062)** | **0.011 (0.005 – 0.025)** | **0.022** |
| C18:1-OH | **0.006 (0.005 – 0.009)** | **0.000 (0.000 – 0.001)** | **0.005** |
| C18:2 | **0.028 (0.019 – 0.034)** | **0.005 (0.003 – 0.015)** | **0.035** |
| C18:2-OH | **0.004 (0.002 – 0.005)** | **0.000 (0.000 – 0.001)** | **0.002** |
| C20 | **0.003 (0.001 – 0.007)** | **0.000 (0.000 – 0.000)** | **0.005** |
| C20:1 | **0.017 (0.008 – 0.030)** | **0.001 (0.000 – 0.003)** | **0.005** |
| C20:2 | **0.013 (0.007 – 0.026)** | **0.001 (0.000 – 0.002)** | **0.005** |
| C20:4 | 0.003 (0.003 – 0.004) | 0.004 (0.002 – 0.006) | 0.945 |
| Total Acylcarnitine (sum) | 0.742 (0.575 – 0.872) | 0.501 (0.189 – 0.776) | 0.101 |

nGDM: placentas from healthy pregnancies (see methods for eligibility criteria); GDM: placentas from gestational diabetes mellitus pregnancies. Relative intensity of fatty-acylcarnitines is expressed as median (p25 – p75). ***a*** Short-chain fatty-acylcarnitines: chain length up to 6 carbons; ***b*** Medium-chain fatty-acylcarnitines: chain length from 8 to 12 carbons; ***c*** Long-chain fatty-acylcarnitines: chain length > 14 carbons.

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***Supplementary figure S5****: Relative abundance of fatty acyl-carnitines identified in the placenta according to mode of delivery vaginal (n=4) or caesarean section (n=3) in the nGDM group*.

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***Supplementary Table 1.*** Internal standards used in the Ion Mobility QTOF LC/MS lipid profile analysis.

oxFA = oxidated fatty acid