

Supplementary material

Fetal exposure to maternal smoking and neonatal metabolite profiles

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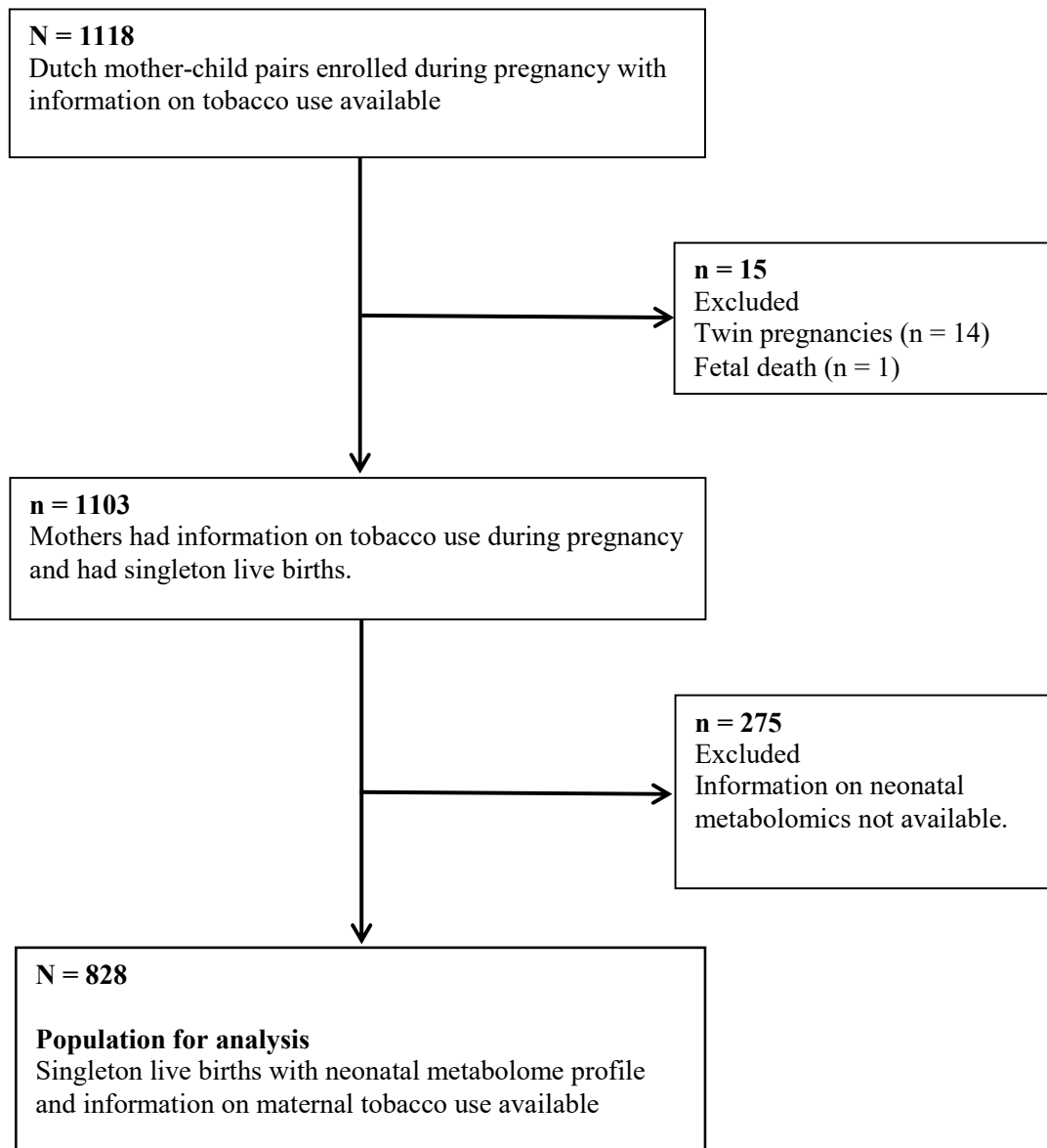
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Figure S1. Flow chart of the study population.



Text S1. Metabolite measurements.

A targeted metabolomics approach was performed at LMU Munich to determinate serum concentrations ($\mu\text{mol/L}$) of amino acids, non-esterified acids (NEFA), phospholipids and Carn, as described previously [1-5]. Proteins of 50 μL serum were precipitated by adding 450 μL methanol following internal standards: labeled amino acid standards set A (NSK-A-1, Cambridge Isotope Laboratories (CIL), USA), 15N2-L-asparagine (NLM-3286-0.25, CIL, USA), indole-D5-L-tryptophan (DLM-1092-0.5, CIL, USA), U-13C16-palmitic acid (CLM-409-MPTPK, CIL, USA), D3-acetyl-carnitine (DLM-754-PK, CIL, USA), D3-octanoyl-carnitine (DLM-755-0.01, CIL, USA) and D3-palmitoyl-carnitine (DLM-1263-0.01, CIL, USA), tridecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (855476, Avanti Polar Lipids, USA) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (850345, Avanti Polar Lipids, USA) [6]. If sample volume was less than optimal, the concentrations were corrected by the respective factor. Sample volumes less than 25 μL were not used and considered missing. After centrifugation, the supernatant was split into aliquots. Amino acids were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described previously [3]. An aliquot of the supernatant was used for the derivatization to amino acids butylester with hydrochloric acid in 1-butanol. After evaporation, the residues were dissolved in water/methanol (80:20; (v/v)) with 0.1% formic acid. The samples were analyzed with 1100 high-performance liquid chromatography (HPLC) system (Agilent, Waldbronn, Germany) equipped with 150 x 2.1 mm, 3.5 μm particle size C18 HPLC column (X-Bridge, Waters, Milford, USA) and 0.1% heptafluorobutyric acid as and ion pair reagent in the mobile phases A and B (A: water, B: methanol). Mass spectrometric (MS) detection was performed with an API2000 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with an atmospheric pressure chemical ionization (APCI) source operating in positive ion ionization mode. IUPAC-IUB Nomenclature was used for notation of the amino acids (1984).

NEFA, phospholipids and Carn were measured with a 1200 SL HPLC system (Agilent, Waldbronn, Germany) coupled to a 4000QTRAP tandem mass spectrometer from AB Sciex (Darmstadt, Germany) [4,7]. NEFA were analyzed by injection of the supernatant to a LC-MS/MS operating in negative electrospray ionization (ESI) mode where they were separated by gradient elution on a 100 x 3.0 mm, 1.9 μm particle size Purusuit UPS Diphenyl column from Varian (Darmstadt, Germany) using 5 mM ammonium acetate in water as mobile phase A and acetonitrile/ isopropanol (80:20; (v/v)) as mobile phase B. NEFA species were quantified using GLC-85 reference standard mixture (Nu-Chek Prep, USA). phospholipids were analyzed by flow-injection analysis (FIA) with LC-MS/MS coupled with ESI [8]. The system was run in positive ionization mode with 5% water in isopropanol as mobile phase A and 5% water in methanol as mobile phase B. The analysis was performed for diacyl-phosphatidylcholines (PC.aa), acyl-alkyl-phosphatidylcholines (PC.ae), acyl- lysophosphatidylcholines (Lyso.PC.a), alkyl-lysophosphatidylcholines (Lyso.PC.e) and sphingomyelins (SM)). Carn (Free carnitine (Free Carn) and acyl-carnitines (Carn.a)) were analyzed by flow-injection analysis of the supernatant into a LC-MS/MS system using an isocratic elution with 76% isopropanol, 19% methanol and 5% water. The mass spectrometer was equipped with electrospray ionization and operated in positive ionization mode. phospholipid and acyl-Carn were quantified using aliquots of a commercial available lyophilized control plasma (ClinChek®, Recipe, Germany), where the concentrations have been determined by AbsoluteIDQ p150 Kit from Biocrates®, a previous published LC-MS/MS method [9] and by in-house quantification with various standards. The analytical process was controlled and post-processed by Analyst 1.6.1. and R Software [6]. The analytical technique used is capable of determining the total number of total bonds, but not the position of the double bonds and the distribution of the carbon atoms between fatty acid side chains. We used the following notation for NEFA, phospholipids and Carn.a: X:Y, where X denotes the length of the carbon chain, and Y the number of double bonds. The ‘a’ denotes an acyl chain bound to the backbone via an ester bond (‘acyl-’) and the ‘e’ represents an ether bond (‘alkyl-’)."

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Table S1. Metabolite ratios.

a) $\sum \text{PC.aa} / \sum \text{PC.ae}$	reflecting oxidative stress
b) $\text{PC.aa.C42:5} / \text{PC.ae.C36:0}$	reflecting oxidative stress
c) $\text{Asn/Asp} / \text{Gln/Glu}$	as indicators for anaplerosis or replenishing of Krebs cycle metabolites
d) Pro/Glu	as marker of proline metabolism
e) $\sum \text{Lyso.PC.a} / \sum \text{PC.aa}$	as a lipid biomarker of inflammation
f) $(\text{LysoPC.a.C18:1} + \text{Lyso.PC.a.C18:2}) / \sum \text{PC.aa}$	as an anti-inflammatory biomarker
g) $\text{Carn.a.C2:0} / \text{Carn.a.C18:0}$	as markers of fatty acid β -oxidation
h) Met/Cys	as a marker of transsulfuration pathway
i) Val/PC.ae.C32:2	as a marker of insulin resistance

Figure S2. Directed acyclic graphic of the study.

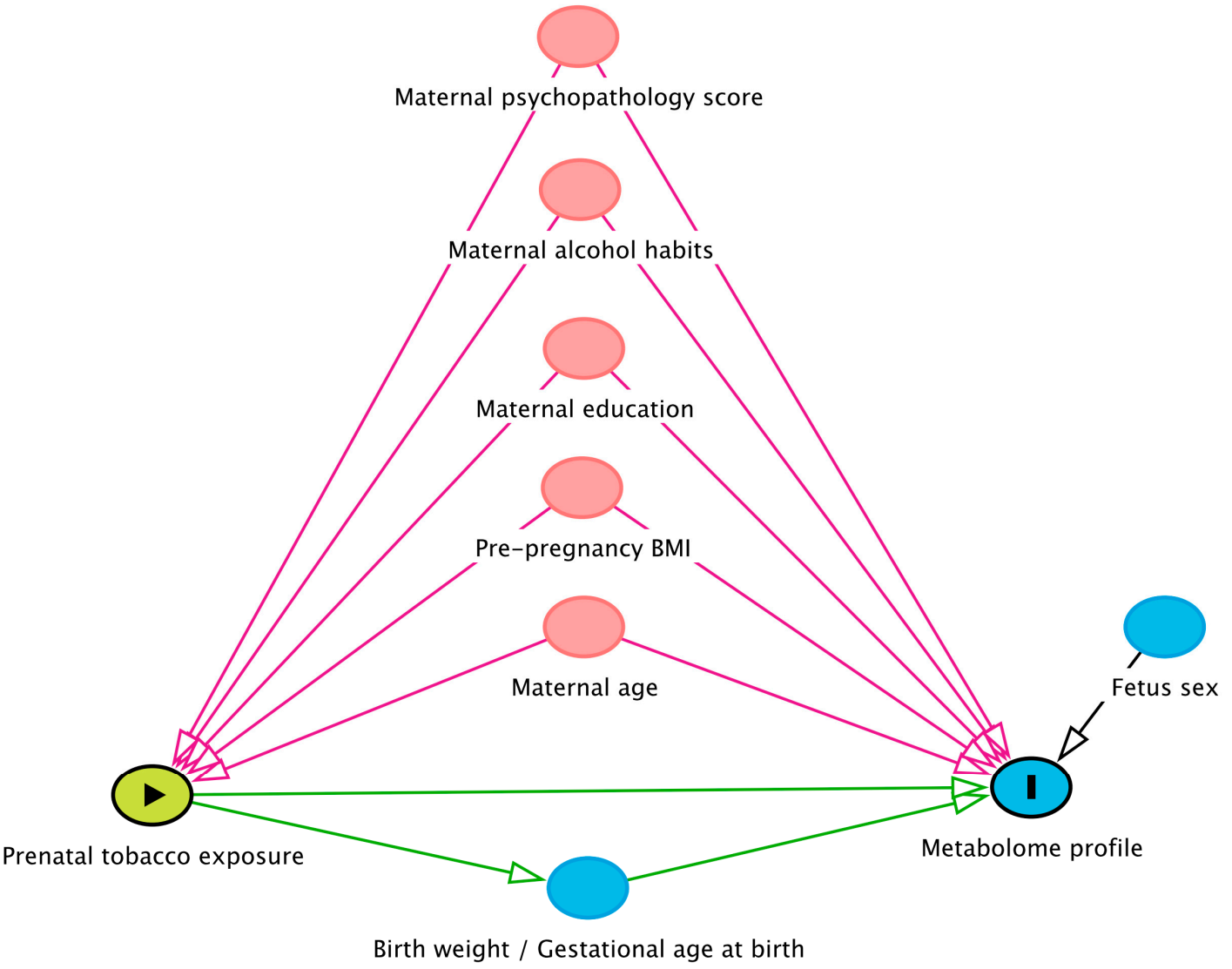


Table S2. Cord blood metabolite concentrations (N = 828).

Neonatal metabolite profile	μmol/L, median (95%)
Amino acids (AA)	3863.99 (2516.72, 5342.86)
BCAA	480.56 (311.22, 710.87)
AAA	257.05 (162.25, 368.40)
Essential AA	1469.32 (997.85, 2034.21)
Non-essential AA	2403.63 (1512.78, 3407.27)
Ala	587.15 (329.51, 1013.83)
Arg	76.15 (30.78, 130.57)
Asn	56.80 (33.80, 95.61)
Asp	40.84 (21.69, 80.8)
Cit	13.92 (8.01, 23.96)
Gln	443.01 (233.6, 824.05)
Glu	196.67 (98.51, 404.77)
Gly	326.11 (211.13, 488.26)
His	132.92 (78.32, 211.73)
Ile	74.78 (44.29, 115.3)
Leu	144.20 (88.77, 228.63)
Lys	340.08 (217.09, 579.05)
Met	33.02 (19.82, 52.87)
Orn	125.00 (71.36, 208.07)
Phe	105.76 (65.36, 157.88)
Pro	193.5 (121.04, 342.79)
Trp	74.99 (45.17, 124.43)
Ser	160.46 (94.61, 298.67)
Thr	270.32 (153.46, 454.51)
Tyr	75.21 (46.14, 115.58)
Val	260.25 (169.64, 384.01)
Cys	16.81 (7.11, 40.76)
Non-esterified fatty acids (NEFA)	189.19 (81.76, 403.67)
Saturated NEFA	90.52 (36.89, 188.43)
Mono-unsaturated NEFA	56.14 (20.93, 133.31)
Poly-unsaturated NEFA	43.69 (19.73, 91.46)
NEFA.14:0	7.08 (2.45, 17.14)
NEFA.14:1	1.53 (0.36, 4.33)
NEFA.15:0	1.04 (0.35, 2.54)
NEFA.16:0	66.60 (27.10, 140.36)
NEFA.16:1	9.85 (2.96, 28.7)
NEFA.16:2	0.46 (0.18, 1.15)
NEFA.17:0	1.09 (0.49, 2.27)
NEFA.17:1	0.52 (0.14, 1.36)
NEFA.17:2	0.03 (0.00, 0.09)
NEFA.18:0	13.97 (3.61, 30.46)
NEFA.18:1	42.24 (16.73, 97.61)
NEFA.18:2	23.94 (9.24, 56.78)
NEFA.18:3	2.06 (0.51, 5.88)
NEFA.19:1	0.15 (0.06, 0.32)
NEFA.20:1	0.42 (0.16, 0.83)

NEFA.20:2	0.55 (0.24, 1.11)
NEFA.20:3	1.76 (0.77, 3.85)
NEFA.20:4	8.13 (3.55, 15.71)
NEFA.20:5	0.28 (0.09, 0.78)
NEFA.22:3	0.13 (0.07, 0.25)
NEFA.22:4	0.62 (0.34, 1.19)
NEFA.22:5	0.74 (0.35, 1.52)
NEFA.22:6	4.15 (1.85, 8.67)
NEFA.24:0	0.17 (0.05, 0.32)
NEFA.24:1	0.17 (0.06, 0.32)
NEFA.24:2	0.09 (0.05, 0.18)
NEFA.24:4	0.12 (0.06, 0.21)
NEFA.24:5	0.10 (0.05, 0.19)
NEFA.26:0	0.14 (0.05, 0.32)
NEFA.26:1	0.09 (0.05, 0.16)
NEFA.26:2	0.06 (0.03, 0.10)

Diacyl-phosphatidylcholines (PC.aa)	750.21 (462.21, 1258.16)
Saturated PC.aa	18.05 (10.75, 32.94)
Mono-unsaturated PC.aa	129.10 (74.39, 236.20)
Poly-unsaturated PC.aa	605.59 (374.78, 1010.75)
PC.aa.C30:0	2.80 (1.43, 5.50)
PC.aa.C30:3	0.13 (0.06, 0.26)
PC.aa.C32:0	11.58 (6.29, 21.85)
PC.aa.C32:1	9.99 (4.81, 22.07)
PC.aa.C32:2	0.69 (0.07, 1.90)
PC.aa.C32:3	0.30 (0.10, 0.60)
PC.aa.C34:1	98.44 (57.24, 178.82)
PC.aa.C34:2	67.48 (38.77, 130.41)
PC.aa.C34:3	2.52 (1.21, 5.28)
PC.aa.C34:4	0.42 (0.19, 0.84)
PC.aa.C34:5	0.05 (0.01, 0.11)
PC.aa.C36:0	1.10 (0.44, 2.11)
PC.aa.C36:1	19.61 (11.20, 35.30)
PC.aa.C36:2	39.07 (22.35, 70.08)
PC.aa.C36:3	66.29 (34.99, 124.57)
PC.aa.C36:4	143.39 (79.60, 239.36)
PC.aa.C36:5	4.59 (2.29, 10.27)
PC.aa.C36:6	0.24 (0.09, 0.51)
PC.aa.C38:0	1.47 (0.66, 3.03)
PC.aa.C38:2	2.85 (1.04, 5.97)
PC.aa.C38:3	42.67 (22.22, 78.41)
PC.aa.C38:4	100.76 (59.51, 173.68)
PC.aa.C38:5	22.15 (12.71, 38.86)
PC.aa.C38:6	69.42 (36.62, 132.21)
PC.aa.C40:0	0.51 (0.21, 1.05)
PC.aa.C40:1	0.22 (0.05, 0.46)
PC.aa.C40:2	0.14 (0.03, 0.37)
PC.aa.C40:3	0.41 (0.14, 0.90)

PC.aa.C40:4	2.90 (1.53, 5.60)
PC.aa.C40:5	6.81 (3.42, 14.16)
PC.aa.C40:6	30.18 (14.16, 58.59)
PC.aa.C42:0	0.58 (0.26, 1.11)
PC.aa.C42:5	0.35 (0.15, 0.67)
PC.aa.C43:6	1.73 (0.86, 3.37)
PC.aa.C44:12	0.30 (0.15, 0.58)

Acyl-alkyl-phosphatidylcholines (PC.ae)	73.29 (47.28, 126.67)
Saturated PC.ae	12.04 (7.22, 20.92)
Mono-unsaturated PC.ae	8.54 (5.11, 15.97)
Poly-unsaturated PC.ae	52.7 (32.93, 92.1)
PC.ae.C30:0	0.23 (0.08, 0.47)
PC.ae.C32:0	2.34 (1.30, 4.49)
PC.ae.C32:1	2.07 (1.06, 4.26)
PC.ae.C32:2	0.44 (0.20, 0.97)
PC.ae.C34:0	0.80 (0.40, 1.74)
PC.ae.C34:1	3.70 (2.03, 7.45)
PC.ae.C34:2	2.42 (1.39, 4.80)
PC.ae.C34:3	1.09 (0.54, 2.34)
PC.ae.C34:4	0.07 (0.02, 0.18)
PC.ae.C36:0	0.54 (0.27, 1.09)
PC.ae.C36:1	1.75 (0.95, 3.43)
PC.ae.C36:2	2.01 (1.14, 3.94)
PC.ae.C36:3	1.90 (1.01, 3.69)
PC.ae.C36:4	8.61 (5.10, 15.93)
PC.ae.C36:5	6.41 (3.53, 12.60)
PC.ae.C38:0	0.95 (0.44, 1.85)
PC.ae.C38:2	0.49 (0.14, 1.15)
PC.ae.C38:3	1.66 (0.84, 3.17)
PC.ae.C38:4	7.23 (4.24, 12.42)
PC.ae.C38:5	7.46 (4.41, 13.63)
PC.ae.C38:6	3.31 (1.98, 6.23)
PC.ae.C40:0	7.1 (3.76, 12.86)
PC.ae.C40:1	0.69 (0.25, 1.39)
PC.ae.C40:2	0.43 (0.05, 1.24)
PC.ae.C40:3	0.47 (0.17, 1.06)
PC.ae.C40:4	1.81 (0.96, 3.38)
PC.ae.C40:5	1.53 (0.85, 2.89)
PC.ae.C40:6	2.13 (1.16, 4.04)
PC.ae.C42:1	0.28 (0.11, 0.55)
PC.ae.C42:3	0.28 (0.08, 0.64)
PC.ae.C42:4	0.53 (0.21, 1.10)
PC.ae.C42:5	1.10 (0.48, 2.33)
PC.ae.C42:6	0.90 (0.41, 1.80)
Acyl-lysophosphatidylcholines (Lyso.PC.a)	144.26 (82.65, 226.65)
Saturated Lyso.PC.a	92.59 (54.22, 150.47)
Mono-unsaturated Lyso.PC.a	17.81 (9.13, 31.49)

Poly-unsaturated Lyso.PC.a	32.38 (16.86, 56.46)
Lyso.PC.a.C14:0	3.25 (1.57, 5.68)
Lyso.PC.a.C16:0	73.43 (42.26, 119.54)
Lyso.PC.a.C16:1	4.73 (2.25, 8.84)
Lyso.PC.a.C18:0	15.32 (9.4, 25.71)
Lyso.PC.a.C18:1	13.09 (6.78, 23.11)
Lyso.PC.a.C18:2	12.00 (5.62, 22.32)
Lyso.PC.a.C18:3	0.29 (0.08, 0.66)
Lyso.PC.a.C20:3	3.83 (1.73, 7.38)
Lyso.PC.a.C20:4	13.32 (6.69, 24.72)
Lyso.PC.a.C20:5	0.27 (0.06, 0.65)
Lyso.PC.a.C22:6	2.43 (1.09, 4.66)
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Alkyl-lysophosphatidylcholines (Lyso.PC.e)	1.65 (0.84, 3.00)
Saturated Lyso.PC.e	1.38 (0.66, 2.61)
Mono-unsaturated Lyso.PC.e	0.28 (0.12, 0.48)
Lyso.PC.e.C16:0	0.55 (0.27, 1.02)
Lyso.PC.e.C18:0	0.84 (0.31, 1.70)
Lyso.PC.e.C18:1	0.28 (0.12, 0.48)
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Sphingomyelins (SM)	220.81 (134.43, 377.50)
Mono-unsaturated SM	106.7 (66.78, 181.17)
Poly-unsaturated SM	112.86 (65.02, 203.72)
SM.a.C30:1	0.13 (0.04, 0.28)
SM.a.C32:1	2.97 (1.62, 5.20)
SM.a.C32:2	0.45 (0.22, 0.86)
SM.a.C33:1	2.18 (1.18, 3.92)
SM.a.C34:1	54.69 (33.74, 96.86)
SM.a.C34:2	11.20 (6.19, 20.05)
SM.a.C35:1	1.66 (0.92, 3.11)
SM.a.C36:1	19.51 (11.01, 32.63)
SM.a.C36:2	12.52 (6.70, 23.42)
SM.a.C36:3	0.36 (0.14, 0.76)
SM.a.C37:1	0.95 (0.45, 1.83)
SM.a.C38:2	6.06 (2.88, 14.02)
SM.a.C38:3	0.20 (0.07, 0.33)
SM.a.C39:1	1.35 (0.61, 2.68)
SM.a.C39:2	0.46 (0.18, 0.83)
SM.a.C40:2	12.03 (5.33, 25.09)
SM.a.C40:5	0.26 (0.13, 0.51)
SM.a.C41:1	4.01 (2.10, 7.70)
SM.a.C41:2	3.42 (1.57, 6.88)
SM.a.C42:1	18.00 (10.58, 32.71)
SM.a.C42:2	35.42 (19.33, 65.21)
SM.a.C42:3	18.09 (8.71, 35.56)
SM.a.C42:4	6.83 (3.66, 12.08)
SM.a.C42:6	2.78 (1.44, 5.32)
SM.a.C43:1	0.95 (0.49, 1.88)
SM.a.C43:2	1.27 (0.65, 2.39)

SM.a.C44:6	1.41 (0.60, 2.54)
SM.e.C36:2	0.24 (0.11, 0.47)
SM.e.C38:3	0.06 (0.02, 0.15)
SM.e.C40:5	0.22 (0.09, 0.42)

Free Carn	16.57 (9.89, 27.67)
Acyl-carnitine (Carn.a)	5.86 (3.56, 10.12)
Short-chain Carn.a	4.47 (2.45, 8.49)
Medium-chain Carn.a	0.52 (0.29, 0.88)
Long-chain Carn.a	0.86 (0.50, 1.34)
Carn.a.C10:0	0.09 (0.04, 0.19)
Carn.a.C10:1	0.08 (0.04, 0.15)
Carn.a.C12:0	0.10 (0.05, 0.18)
Carn.a.C14:1	0.05 (0.02, 0.12)
Carn.a.C14:2	0.04 (0.01, 0.08)
Carn.a.C15:0	0.04 (0.02, 0.07)
Carn.a.C16:0	0.16 (0.09, 0.28)
Carn.a.C16:0.Oxo	0.02 (0.01, 0.04)
Carn.a.C16:1	0.11 (0.05, 0.20)
Carn.a.C16:2	0.03 (0.02, 0.07)
Carn.a.C18:0	0.09 (0.05, 0.15)
Carn.a.C18:1	0.09 (0.05, 0.17)
Carn.a.C18:2	0.07 (0.03, 0.12)
Carn.a.C18:2.OH	0.02 (0.01, 0.04)
Carn.a.C2:0	3.75 (1.93, 7.37)
Carn.a.C20:0	0.03 (0.02, 0.05)
Carn.a.C20:1	0.00 (0.00, 0.00)
Carn.a.C20:3	0.06 (0.03, 0.10)
Carn.a.C20:4	0.00 (0.00, 0.01)
Carn.a.C3:0	0.31 (0.17, 0.61)
Carn.a.C3:0.DC	0.11 (0.04, 0.32)
Carn.a.C4:0	0.14 (0.08, 0.26)
Carn.a.C5:0	0.14 (0.06, 0.32)
Carn.a.C6:0	0.05 (0.02, 0.12)
Carn.a.C6:0.OH	0.04 (0.02, 0.10)
Carn.a.C8:0	0.06 (0.02, 0.13)
Carn.a.C8:1	0.05 (0.02, 0.12)
Carn.a.C9:0	0.02 (0.01, 0.04)

Values are presented as medians (95% range) of neonatal metabolites concentration in cord blood (μmol/L)

Table S3. Non-response analysis.

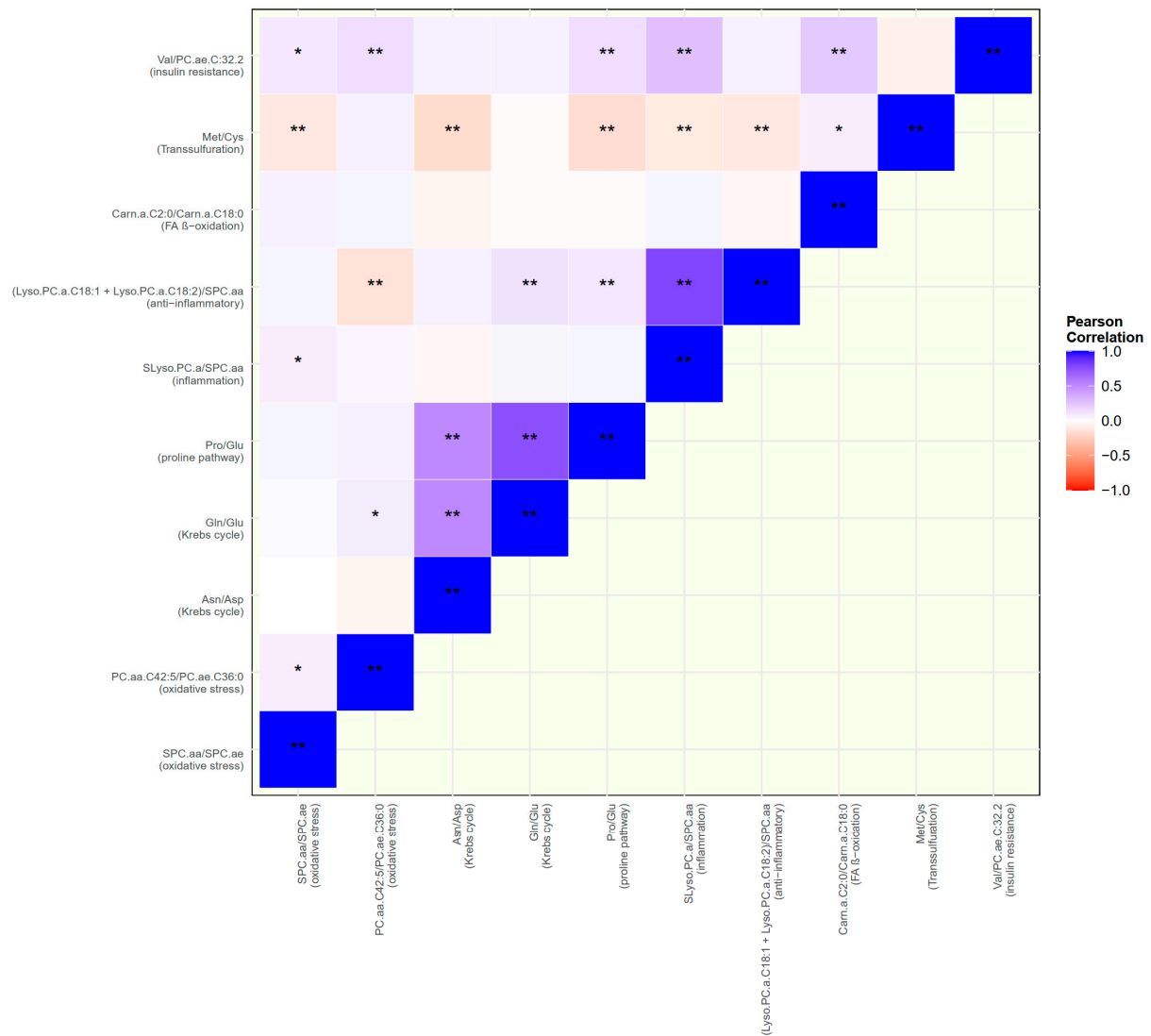
	Participants with information on neonatal metabolomics n = 828	Participants without information on neonatal metabolomics n = 275	p-value*
Maternal characteristic			
Age, years, mean (\pm SD)	31.4 (4.1)	31.5 (4.3)	0.71
Missing (%)	-	-	
Educational level			
None/Primary (%)	2.3	2.2	0.98
Secondary (%)	35.3	35.3	
Higher (%)	62.1	60.4	
Missing (%)	0.4	2.2	
Maternal alcohol use			
Never during pregnancy (%)	31.3	31.3	0.99
First trimester only (%)	15.2	14.9	
Continued pregnancy (%)	51.1	49.8	
Missing (%)	2.4	4.0	
Pre-pregnancy BMI, kg/m ² , median (95% range)	22.5 (18.4 – 33.5)	22.6 (18.5 – 37.8)	0.46
Missing (%)	12.2	8.4	
Psychopathology score, median (95% range)	0.1 (0.0 – 0.7)	0.1 (0.0 – 0.7)	0.75
Missing (%)	6.5	6.2	
Birth characteristic			
Female sex, yes (%)	46.1	53.5	0.03
Missing (%)	-	0.4	
Gestational age, weeks, median (95% range)	40.3 (36.7 – 42.3)	40.1 (32.8 – 42.4)	0.08
Birth weight, gram, mean (\pm SD)	3549.2 (506.6)	3407.7 (658.6)	<0.01
Missing (%)	0.1	0.4	

Values are means and standard deviation (\pm SD), medians (95% range), or percentages.

*Differences in subjects' characteristics between the groups were evaluated using T-test for the normally distributed continuous variables, Man Whitney-U test for the not-normally distributed continuous variables, and chi-square tests for proportions.

Abbreviations: SD: standard deviation

Figure S3. Correlations between selected neonatal metabolite ratios.

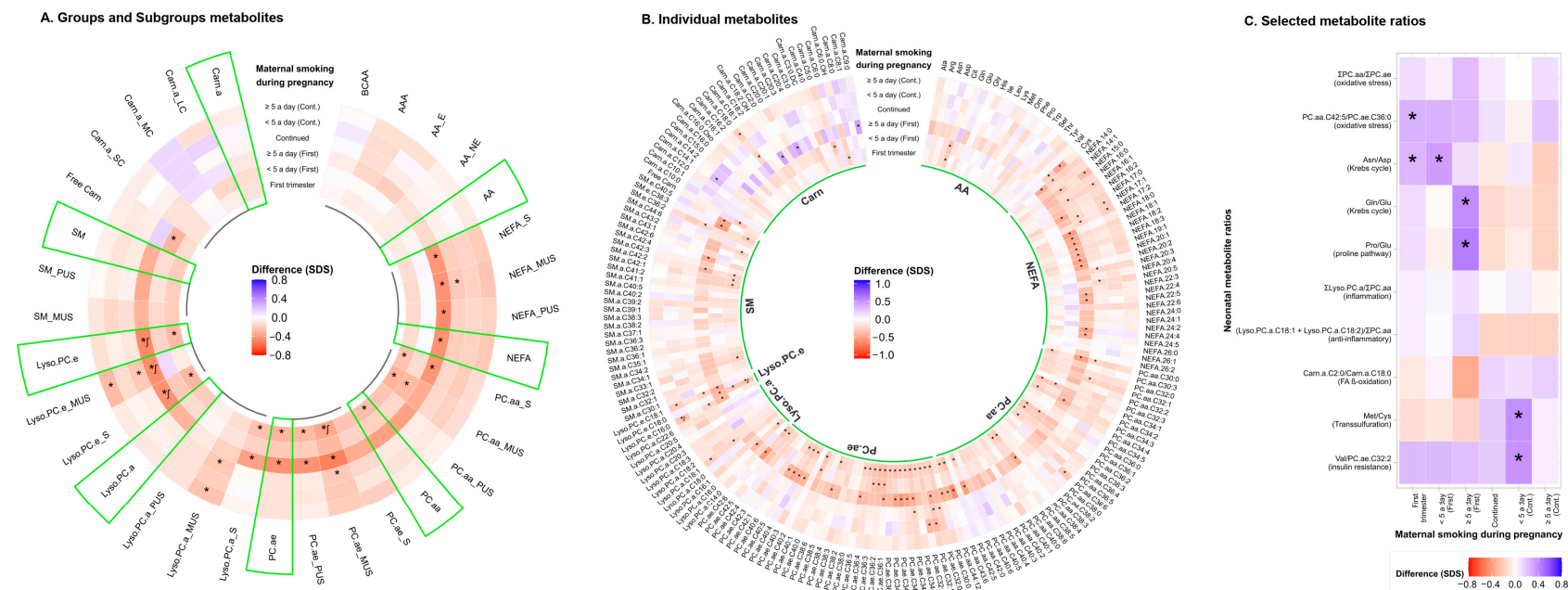


Values represent Pearson's correlation coefficient between selected neonatal metabolite ratios square root transformed.

Abbreviations: PC.aa, diacyl-phosphatidylcholines; PC.aa, acyl-alkyl-phosphatidylcholines; Lyso.PC.a, acyl-lysophosphatidylcholines; Carn.a, acyl-carnitines; Asn/Asp, asparagine/aspartic acid; Gln/Glu, glutamine/glutamic acid; Pro/Glu, proline/glutamic acid; FA β-oxidation, Fatty acid β-oxidation; Met/Cys, methionine/cysteine; and Val, valine.

* p-value <0.05, ** p-value <0.01.

Figure S4. Associations of maternal smoking during pregnancy with cord blood metabolite profile and ratios adjusted for birth weight.



Values represent regression coefficients based on linear models that reflect the positive (blue) and negative (red) difference in neonatal cord blood metabolite concentrations and ratios in standard deviation scores in neonates whose mothers smoked any time of pregnancy compared to mothers who did not smoke. Models were adjusted for maternal age, educational level, alcohol consumption, pre-pregnancy BMI, psychopathology symptoms, fetal sex, and birth weight (birth model).

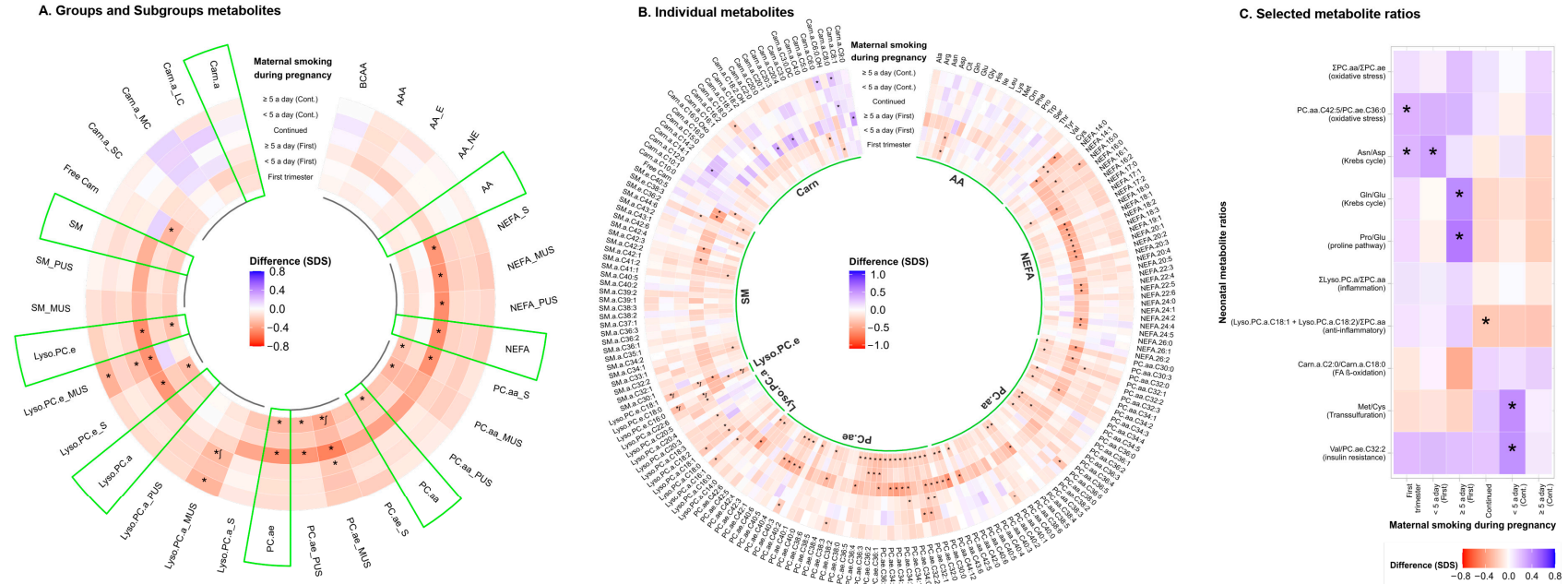
* p-value < 0.05.

† Significant associations after FDR corrected p-value.

The sectors of green color showed group of neonatal metabolite profile, which is preceded by metabolite profile subgroups.

Abbreviations: SDS, standard deviation scores; BMI, body mass index; AA, amino acids; BCAA, branched-chain AA; AAA, aromatic AA; AA_E, essential AA; AA_NE, non-essential AA; NEFA, non-esterified fatty acids; NEFA_S, saturated NEFA; NEFA_MUS, mono-unsaturated NEFA; NEFA_PUS, poly-unsaturated NEFA; PC.aa, diacyl-phosphatidylcholines; PC.aa_S, saturated PC.aa; PC.aa_MUS, mono-unsaturated PC.aa; PC.aa_PUS, poly-unsaturated PC.aa; PC.ae, acyl-alkyl-phosphatidylcholines; PC.ae_S, saturated PC.ae; PC.ae_MUS, mono-unsaturated PC.ae; PC.ae_PUS, poly-unsaturated PC.ae; Lyso.PC.a, acyl-lysophosphatidylcholines; Lyso.PC.a_S, saturated Lyso.PC.a; Lyso.PC.a_MUS, mono-unsaturated Lyso.PC.a; Lyso.PC.a_PUS, poly-unsaturated Lyso.PC.a; Lyso.PC.e, alkyl-lysophosphatidylcholines; Lyso.PC.e_S, saturated Lyso.PC.e; Lyso.PC.a_MUS, mono-unsaturated Lyso.PC.e; SM, sphingomyelins; SM_MUS, mono-unsaturated SM; SM_PUS, poly-unsaturated SM; Free Carn, free carnitine; Carn.a, acyl-carnitines; Carn.a_SC, short-chain Carn.a; Carn.a_MC, medium-chain Carn.a; and Carn.a_LC, long-chain Carn.a; Asn/Asp, asparagine/aspartic acid; Gln/Glu, glutamine/glutamic acid; Pro/Glu, proline/glutamic acid; FA β-oxidation, Fatty acid β-oxidation; Met/Cys, methionine/cysteine; and Val, valine.

Figure S5. Associations of maternal smoking during pregnancy with cord blood metabolite profile and ratios adjusted for gestational age.



Values represent regression coefficients based on linear models that reflect the positive (blue) and negative (red) difference in neonatal cord blood metabolite concentrations and ratios in standard deviation scores in neonates whose mothers smoked any time of pregnancy compared to mothers who did not smoke. Models were adjusted for maternal age, educational level, alcohol consumption, pre-pregnancy BMI, psychopathology symptoms, fetal sex, and gestational age (birth model).

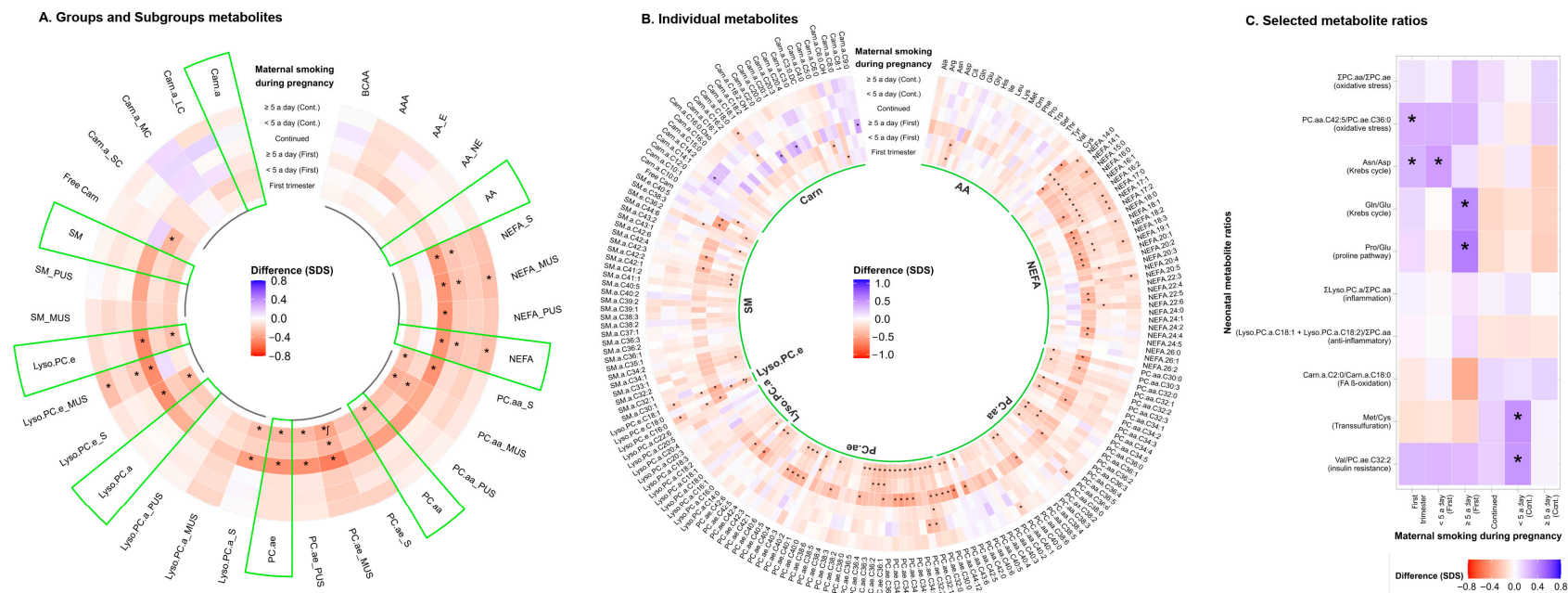
* p-value < 0.05.

† Significant associations after FDR corrected p-value.

The sectors of green color showed group of neonatal metabolite profile, which is preceded by metabolite profile subgroups.

Abbreviations: SDS, standard deviation scores; BMI, body mass index; AA, amino acids; BCAA, branched-chain AA; AAA, aromatic AA; AA_E, essential AA; AA_NE, non-essential AA; NEFA, non-esterified fatty acids; NEFA_S, saturated NEFA; NEFA_MUS, mono-unsaturated NEFA; NEFA_PUS, poly-unsaturated NEFA; PC:aa, diacyl-phosphatidylcholines; PC:aa_S, saturated PC:aa; PC:aa_MUS, mono-unsaturated PC:aa; PC:aa_PUS, poly-unsaturated PC:aa; PC:ae, acyl-alkyl-phosphatidylcholines; PC:ae_S, saturated PC:ae; PC:ae_MUS, mono-unsaturated PC:ae; PC:ae_PUS, poly-unsaturated PC:ae; Lyso:PC:aa, acyl-lysophosphatidylcholines; Lyso:PC:aa_S, saturated Lyso:PC:aa; Lyso:PC:aa_MUS, mono-unsaturated Lyso:PC:aa; Lyso:PC:aa_PUS, poly-unsaturated Lyso:PC:aa; Lyso:PC:ae, alkyl-lysophosphatidylcholines; Lyso:PC:ae_S, saturated Lyso:PC:ae; Lyso:PC:ae_MUS, mono-unsaturated Lyso:PC:ae; SM, sphingomyelins; SM_MUS, mono-unsaturated SM; SM_PUS, poly-unsaturated SM; Free Carn, free carnitine; Carn:aa, acyl-carnitines; Carn:aa_SC, short-chain Carn:aa; Carn:aa_MC, medium-chain Carn:aa; and Carn:aa_LC, long-chain Carn:aa; Asn/Asp, asparagine/aspartic acid; Gln/Glu, glutamine/glutamic acid; Pro/Glu, proline/glutamic acid; FA β-oxidation, Fatty acid β-oxidation; Met/Cys, methionine/cysteine; and Val, valine.

Figure S6. Associations of maternal smoking during pregnancy with cord blood metabolite profile and ratios adjusted for gestational age adjusted birth weight.



Values represent regression coefficients based on linear models that reflect the positive (blue) and negative (red) difference in neonatal cord blood metabolite concentrations and ratios in standard deviation scores in neonates whose mothers smoked any time of pregnancy compared to mothers who did not smoke. Models were adjusted for maternal age, educational level, alcohol consumption, pre-pregnancy BMI, psychopathology symptoms, fetal sex, and Niklasson standard (birth model).

* p-value < 0.05.

† Significant associations after FDR corrected p-value.

The sectors of green color showed group of neonatal metabolite profile, which is preceded by metabolite profile subgroups.

Abbreviations: SDS, standard deviation scores; BMI, body mass index; AA, amino acids; BCAA, branched-chain AA; AAA, aromatic AA; AA_E, essential AA; AA_NE, non-essential AA; NEFA, non-esterified fatty acids; NEFA_S, saturated NEFA; NEFA_MUS, mono-unsaturated NEFA; NEFA_PUS, poly-unsaturated NEFA; PC.aa, diacyl-phosphatidylcholines; PC.aa_S, saturated PC.aa; PC.aa_MUS, mono-unsaturated PC.aa; PC.aa_PUS, poly-unsaturated PC.aa; PC.ae, acyl-alkyl-phosphatidylcholines; PC.ae_S, saturated PC.ae; PC.ae_MUS, mono-unsaturated PC.ae; PC.ae_PUS, poly-unsaturated PC.ae; Lyso.PC.a, acyl-lysophosphatidylcholines; Lyso.PC.a_S, saturated Lyso.PC.a; Lyso.PC.a_MUS, mono-unsaturated Lyso.PC.a; Lyso.PC.a_PUS, poly-unsaturated Lyso.PC.a; Lyso.PC.e, alkyl-lysophosphatidylcholines; Lyso.PC.e_S, saturated Lyso.PC.e; Lyso.PC.a_MUS, mono-unsaturated Lyso.PC.e; SM, sphingomyelins; SM_MUS, mono-unsaturated SM; SM_PUS, poly-unsaturated SM; Free Carn, free carnitine; Carn.a, acyl-carnitines; Carn.a_SC, short-chain Carn.a; Carn.a_MC, medium-chain Carn.a; and Carn.a_LC, long-chain Carn.a; Asn/Asp, asparagine/aspartic acid; Gln/Glu, glutamine/glutamic acid; Pro/Glu, proline/glutamic acid; FA β-oxidation, Fatty acid β-oxidation; Met/Cys, methionine/cysteine; and Val, valine.