TECHNISCHE UNIVERSITÄT MÜNCHEN

TUM School of Life Sciences

Lehrstuhl für Ernährungsphysiologie

Metabolic profiles in umbilical cord blood in response to maternal obesity and in relation to offspring longitudinal weight development

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Vollständiger Abdruck der von der Fakultät TUM School of Life Sciences der Technischen Universität München zur Erlangung des akademischen Grades

eines Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

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Prüfer der Dissertation:

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Die Dissertation wurde am 26.10.2020 bei der Technischen Universität München eingereicht und durch die Fakultät TUM School of Life Sciences am 17.03.2021 angenommen.

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ABC = ATP binding cassette

ABCG1 = ATP Binding Cassette Subfamily G Member 1

ACADM = acyl-CoA dehydrogenase medium chain

ACADS = acyl-CoA dehydrogenase short chain

ACADVL = very long chain acyl-CoA dehydrogenase

ACAD8 = acyl-CoA dehydrogenase family member 8

AdipoRiSc = Adiposity Risk Screening

ALDH7A1 = aldehyde dehydrogenase 7 family member A1

BIA = bioelectrical impedance analysis

BCAA = branched-chain amino acids

BCAT1 = branched-chain amino acid transaminase 1

BCKDK = branched chain ketoacid dehydrogenase kinase

BCKDHA = branched chain keto acid dehydrogenase E1, alpha polypeptide

BMI = Body mass index

CDC = Center for Disease Control and Prevention

cDNA = complementary DNA

CI = confidence interval

CK = choline kinase

CMP = cytidine monophosphate

CoA = coenzyme A

CPD = cytidine 5'-diphosphocholine

CPT = CDP-choline:1,2-diacylglycerol cholinephosphotransferase

CPT1 = carnitine palmitoyltransferase I;

CPT2 = carnitine palmitoyltransferase II;

CRAT = carnitine O-acytelytransferase

CROT = carnitine O-octanoyltransferase

CRP = C-reactive protein

CV = Coefficient of variation

CT = CTP-phosphocholine cytidylyltransferase

C-peptide = connecting peptide

C-section = cesarean section

DLD = dihydrolipoamide dehydrogenase

DNMT = DNA methyltransferase

DXA = dual energy X-ray absorptiometry

EDTA = Ethylenediaminetetraacetic acid

ELISA = enzyme-linked immunosorbent assay

ER = estrogen receptor

FA = fatty acid

FABPpm = plasma membrane fatty acid binding protein

FAT = fatty acid translocase

FATP = fatty acid transport protein

FXR = farnesoid x receptor

GA = gestational age

GCT = glucose challenge test

GDM = gestational diabetes mellitus

gDNA = genomic DNA

GLUT = glucose transporter

GWG = gestational weight gain

HAPO = Hyperglycemia and Adverse Pregnancy Outcome

HbA1c = glycated hemoglobin

HDL= high-density lipoprotein

HPLC = High performance liquid chromatography

HPRT1 = hypoxanthine phosphoribosyltransferase 1

IOM = Institute of medicine

IQTIG = Institute for Quality Assurance and Transparency in Healthcare

IVD = isovaleryl-CoA dehydrogenase

KiGGS = German Health Interview and Examination Survey for Children and Adolescents

LCAT = lecithin-cholesterol acyltransferase

LC-MS/MS=liquid chromatography coupled to tandem mass spectrometry

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

LGA = large-for-gestational age

LOD = limit of detection

LOQ = limit of quantification

LPCAT = lysophosphatidylcholine acyltransferase

MAT = methionine adenosyltransferase

MRM = multiple reaction monitoring

mTOR = mechanistic target of rapamycin

MTR = 5-methyltetrahydrofolate-homocysteine methyltransferase

OAT = organo anion

OCNT2 = organic cation/carnitine transporter 2

OD = optical density

OGTT = oral glucose tolerance test

OPLS = orthogonal partial latent structure

OR = odds ratio

PC = phosphatidylcholine

PCR = polymerase chain reaction

PEACHES = Programming of Enhanced Adiposity Risk in CHildhood - Early Screening

PEMT = phosphatidylethanolamine N-methyltransferase

PROEBE = Prospective randomized open blinded end-point

PLA = phospholipase A

PLS-DA = partial latent structure-discriminant analysis

RKI = Robert Koch Institute

RT = room temperature

RT-PCR = reverse transcription polymerase chain reaction

qRT-PCR = real-time quantitative reverse transcription polymerase chain reaction

RR = relative risk

SD = standard deviation

SE = standard error

SEM = standard error of the mean

SES = socio-economic status

SHMT = serine hydroxymethyltransferase

SLC25A20 = solute carrier family 25 member 20

SM = sphingomyelin

SMase = sphingomyelin phosphodiesterase

SMS = sphingomyelin synthase

SRBI = scavemger receptor class B type I

TG = triglyceride

T2D = type 2 diabetes

UBE2D2 = ubiquitin conjugating enzyme E2 D2

UCB = umbilical cord blood

US = United States

UPBEAT = UK Pregnancies Better Eating and Activity Trial

VLDL = very low-density lipoprotein

VLDLR = very low-density lipoprotein receptor

WHO = World Health Organization

5-mC = 5-methylcytosin

Summary

Globally, prevalence rates of overweight/obesity have increased dramatically over the past decades. Prevalences of children and women of childbearing age are of particular concern. Maternal preconception obesity can alter the intrauterine milieu and predispose the offspring to adverse short and long-term health consequences via mechanisms of fetal programming. However, the underlying processes and related metabolic pathways of this vicious cycle remain unclear.

The main aim of this thesis was to analyze the influence of maternal preconception obesity on the metabolite profile, metabolite concentrations and metabolic pathways in umbilical cord blood (UCB) and the relationship with child BMI outcome by means of the prospective cohort Programming of Enhanced Adiposity Risk in Childhood-Early Screening (PEACHES). Further, we aimed to compare metabolite concentrations in UCB of offspring of normal weight mothers to those of female adult blood. In addition, we analyzed the effect of maternal late-pregnancy dysglycemia on the UCB metabolite profile in a subgroup of preconceptionally obese women. PEACHES enrolled n = 1707 mother-child pairs to investigate short- and long-term effects of preconception obesity and gestational diabetes mellitus (GDM) on mother and child. Using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), 209 metabolites were quantified in UCB of n = 398 offspring of preconception normal weight (n = 111), obese (n = 128) and severely obese mothers (n = 159). Gene expression and global methylation were analyzed in smaller subgroups. Statistical analyses included hierarchical clustering, multiple linear regression models, and linear mixed effect models. Compared to adult blood, UCB differed in metabolite concentrations showing e.g. higher concentrations of the vast majority of amino acid and lower concentrations of medium-chain acyl-carnitines. The UCB metabolite profile differed depending on the severity of maternal preconception obesity. Altered metabolite concentrations were found in relation to the branched chain amino acid (BCAA) catabolism and the one-carbon metabolism in offspring of preconception severely obese vs. offspring of normal weight mothers. These alterations were confirmed by downregulated mRNA expression of genes involved in these pathways. Downregulated mRNA expression was in line with a global hypermethylation of UCB of offspring of preconception severely obese mothers. Findings in UCB of offspring of preconceptionally obese mothers with late-pregnancy dysglycemia suggested an increased β-oxidation in UCB through an exposure to a dysglycemic environment in addition to obesity. The relationship between UCB metabolites and measures of child BMI development at preschool age was dependent on maternal preconception BMI and offspring sex.

In conclusion, metabolite alterations at birth related to maternal preconception obesity may partly reflect adaptive fetal processes which might have long-term, possibly sex-specific health consequences for the offspring.

Zusammenfassung

Die Prävalenz von Übergewicht/Adipositas ist in den letzten Jahrzehnten weltweit drastisch angestiegen. Hohe Raten von Frauen im gebärfähigen Alter und Kindern sind besonders beunruhigend. Präkonzeptionelle Adipositas kann das Milieu in utero verändern und Nachkommen über Mechanismen der fetalen Programmierung für adverse Kurz- und Langzeitfolgen prädisponieren. Zugrunde liegende Prozesse und Stoffwechselwege sind weitestgehend unklar.
Das Hauptziel war es, den Einfluss von maternaler präkonzeptioneller Adipositas auf das Metabolitenprofil und Stoffwechselprozesse im Nabelschnurblut (NSB) sowie den Zusammenhang mit dem kindlichen BMI-Outcome anhand der *Programming of Enhanced Adiposity Risk in Childhood-Early Screening* (PEACHES) - Studie zu untersuchen. Weiterhin wurden Metabolitenkonzentrationen im NSB von Nachkommen normalgewichtiger Mütter und adultem Blut verglichen und der Einfluss von Dysglykämien in der adipösen Spätschwangerschaft auf das Metabolitenprofil im NSB untersucht. PEACHES besteht aus n = 1707 Mutter-Kind-Paaren, die rekrutiert wurden, um die kurz- und langfristigen Auswirkungen von maternaler präkonzeptioneller Adipositas und Schwangerschaftsdiabetes (GDM) auf Mutter und Kind zu untersuchen. Mittels Liquid-Chromatographie-Massenspektrometrie/-Massenspektrometrie wurden 209 Metaboliten im NSB von n = 398 Nachkommen präkonzeptionell normalgewichtiger (n = 111), adipöser (n = 128) und stark adipöser Mütter (n = 159) quantifiziert. Genexpression und globale Methylierung wurden im NSB untersucht. Statistische Analysen beinhalteten u.a. lineare multiple Regressionsmodelle und lineare gemischte Modelle. Verglichen mit adultem Blut wies das NBS andere Metabolitenkonzentrationen, wie höhere Konzentrationen der meisten Aminosäuren und geringere Konzentrationen von mittelkettigen Acyl-carnitinen, auf. Das Metabolitenprofil im NSB unterschied sich gemäß dem Schweregrad der maternalen präkonzeptionellen Adipositas. Der Vergleich des NSB von Nachkommen präkonzeptionell normalgewichtiger vs. stark adipöser Mütter zeigte veränderte Konzentrationen in Abbauprodukten der verzweigtkettigen Aminosäuren (BCAA) und Intermediate des Ein-Kohlenstoff-Metabolismus gefunden. Diese Veränderungen gingen mit einer reduzierten mRNA-Expression ausgewählter Gene dieser Stoffwechselwegen und einer globalen Hypermethylierung des NSB einher. Weitere Ergebnisse deuteten auf eine erhöhte β-Oxidation im NSB nach Exposition gegenüber einer dysglykämischen Umgebung zusätzlich zur einer maternalen Adipositas in der Schwangerschaft hin. Die Zusammenhänge zwischen NSB-Metaboliten und der BMI Entwicklung der Kinder waren abhängig vom BMI der Mutter und Geschlecht der Nachkommen.
Mit maternaler präkonzeptioneller Adipositas einhergehende Metabolitenveränderungen bei Geburt spiegeln möglicherweise adaptive fetale Prozesse wider, die potentiell auch langfristige, vermutlich geschlechtsspezifische gesundheitliche Folgen für die Nachkommen haben könnten.

1 Introduction

1.1 Obesity – a global public health problem

Since 1975, obesity prevalence rates have almost tripled (1). According to the World Health Organization (WHO), in 2016, more than 1.9 billion adults were overweight, of which 650 million were obese. Obesity is a multifactorial medical condition, which adversely affects the individual´s health by increasing the risk for developing comorbidities such as metabolic and cardiovascular diseases. Obesity causes more deaths worldwide than underweight and has a significant, negative impact on the economy (1).

1.1.1 Definition of obesity

Obesity is defined as an abnormal or excessive fat accumulation that may cause health impairments (2). The body fat proportion and its distribution are major risk factors for related adverse health consequences, which differ inter-individually by ethnicity, age and sex (3). However, the exact measurement of body fat proportion and its distribution including bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DXA) is rather difficult and costly. Even though the body mass index (BMI) does not measure body composition directly, it is widely used as a screening tool to determine overweight and obesity, estimate obesity prevalence rates and identify risk groups in adults (3), as it correlates well with body fat proportion (4). The BMI is defined as a person's weight in kilograms divided by the squared height in meters (kg/m^2) and categorizes a person with a BMI $\geq$ 30.0 kg/m^2 as obese, according to the WHO. All BMI categories for adults according to the WHO are displayed in Table 1 (3).

Table 1: BMI classification for adults according to the WHO (3)

Classification	BMI (kg/m^2)
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	≥25.0
Preobesity	25.0-29.9
Obesity	≥30.0
Obese class I	30.0-34.9
Obese class II	35.0-39.9
Obese class III	≥40.0

BMI, body mass index.

In childhood and adolescence, age- and sex-specific BMI percentiles or BMI z-scores are used to account for constant changes in height and body composition. Depending on age, sex and ethnicity, these changes occur at different times and rates, including the onset of puberty and interindividual rates of fat accumulation (3). BMI reference percentiles for German children are provided by the Robert Koch Institute (RKI) based on the nationwide survey 'German Health

Interview and Examination Survey for Children and Adolescents' (KiGGS) (5, 6). Children with a BMI ≥ 90th percentile are classified as overweight, those with a BMI ≥ 97th percentile as obese (5, 6). BMI z-scores are transnationally based on WHO references (7). Children until the age of five years are categorized as overweight when the BMI z-score is >+2 standard deviations (SD) and obese when >+3 SD (8). Children beyond the age of five years are categorized as overweight when the BMI z-score is >+1 SD and obese when >+2 SD (8). The classification of childhood BMI is not as straight forward as the one for adults and different classifications for overweight and obesity are used throughout the literature. The term "overweight/obesity" is used regardless of the exact classification to stress the fact that, as for adults, obese children are included in the group of overweight children.

1.1.2 Prevalence of overweight and obesity

Over the past decades, the prevalence rates of overweight and obesity have increased dramatically, worldwide, and throughout all age groups (9-11). Since 1975, global childhood obesity rates have increased tenfold (9). In the adult population, prevalence rates of overweight and obesity have increased by 50 % and 80 %, respectively, over 35 years (11). By 2015, 39 % of the world's population was estimated to be overweight, including 12.5 % obese. Prevalence rates in the United States (US) and Europe, including Germany, were even more alarming: about two thirds of the US and more than half of the European adult population, were overweight – one third and one fifth of these were obese, respectively (11). Even though higher obesity rate are generally attributed to an older proportion of the population, 37% of US women of childbearing age (20 - 39 years) were found to be obese (12). Recent evaluations of the RKI confirmed rising overweight and obesity rates in Germany likewise, especially in younger adults, declaring about one third of women of childbearing age (18 - 49 years) as overweight, including about 15 % to be obese (13, 14). Correspondingly, data of the German Institute for Quality Assurance and Transparency in Healthcare (IQTIG) reported one in six women to be obese at their first antenatal visit at the gynecologist in Germany (15). Along with this, in Germany, every sixth child was overweight or obese according to the latest results of the KiGGS study (16).

Maternal obesity at conception is a well-known risk factor for the development of childhood obesity (17), which in turn increases the risk for obesity in adulthood for the offspring (18). Thus, high rates of overweight and obesity in women of childbearing age and children are especially worrisome and support the idea of a transgenerational, 'vicious cycle' of obesity (19).

1.2 Maternal obesity – consequences for mother and child

The term “maternal preconception obesity” refers to women of childbearing age that enter pregnancy with excessive body weight (BMI ≥ 30.0 kg/m^2) (20-24). During this period, obesity is of particular concern since maternal preconception obesity is associated with several complications prior to and during pregnancy and bears adverse short- and long-term health consequences for both, mother and offspring (20-24).

1.2.1 Pre- and perinatal outcomes

Maternal preconception obesity has been linked to infertility and several pre- and perinatal complications including gestational diabetes mellitus (GDM), pre-eclampsia, miscarriage, and high birthweight (20, 21). The adverse outcomes are often interrelated and the risks for developing obesity-related health complications increase with obesity class (20, 21).

The risk for developing GDM is considerably higher in preconception obese women than in normal weight women (odds ratio (OR) (95% confidence interval (CI)) for women with obesity class I, II, and III: 2.94 (2.73 – 3.18), 3.97 (3.61 – 4.36), and 5.47 (4.96 – 7.21), respectively) (25). The reasons for this increased risk have not been fully elucidated. However, an excess of inflammatory markers produced by an abundance of adipocytes and an exacerbation of the physiological insulin resistance during pregnancy in obese women contributing to maternal hyperglycemia and dyslipidemia are discussed as potential causes (26). Alterations in metabolic functions, such as insulin resistance and chronic low-grade inflammation as well as endothelial dysfunction, oxidative stress, and failure of invasion of the trophoblast cells, have further been linked to an increased risks of pre-eclampsia (relative risk (RR) (CI 95%) for women with obesity class I and II/III combined: 2.93 (2.58 – 3.33) and 4.14 (3.61 – 4.75), respectively) compared to normal weight women (27). Pre-eclampsia is a condition characterized by substantial proteinuria (0.3g/24 hours) and hypertension during pregnancy (persistent diastolic blood pressure > 90 mmHg) (28), which itself is a pregnancy complication in obese women (OR (95% CI): 4.5 (4.1 – 5.0)) (29). Rates of antenatal depression and anxiety are higher in preconception obese compared to normal weight women (OR (95% CI): 1.43 (1.27-1.61) and 1.41 (1.10 - 1.80), respectively) (30).

Further, there is an increased risk of congenital anomalies in obese compared to normal pregnancies, such as neural tube defects (OR (95% CI): 1.87 (1.62 – 2.15)), which were linked to undiagnosed diabetes and malnutrition in obese mothers (31). Compared to normal weight women, obese women have a higher risk of miscarriage (OR (95% CI) for women with obesity class I, II, and III: 1.34 (1.22 - 1.47), 1.97 (1.71 - 2.28), and 3.54 (2.56 - 4.89), respectively) and stillbirth (OR (95% CI) for women with obesity class I, II, and III: 1.46 (1.37 – 1.55), 1.78 (1.67 – 1.91), and 2.19 (2.03 – 2.36), respectively), which may be related to pregnancy

complications including those previously mentioned (32). Additionally, overnutrition and lack of physical activity in obese women are related to a higher likelihood to experience excessive gestational weight gain (GWG) (OR (95% CI): 2.5 (1.8–3.5)) (33). According to the Institute of Medicine (IOM), adequate weight gain during pregnancy is related to a woman's preconception BMI, where obese women should have a lower total weight gain during pregnancy as normal weight woman (34). The adequate weight gain as proposed by the IOM is depicted in Table 2. Lower weight gain would be classified as inadequate, higher weight gain as excessive (34).

Table 2: Recommended total GWG by the IOM (34)

Preconception BMI (kg/m^2)	Total GWG (kg)
<18.5	12.5-18.0
18.5-24.9	11.5-16.0
25.0-29.9	7.0-11.5
≥30.0	5.0-9.0

BMI, body mass index; GWG, gestational weight gain; IOM, Institute of Medicine

Maternal overnutrition, affecting fetal structure, physiology and metabolism in preconception obese women, was further linked to a higher risk for a high birth weight (>4000 g), large-for-gestational age (LGA, >90th percentile) infants, and macrosomia, a birthweight >4500 g (OR (95% CI): 2.00 (1.84 – 2.18), 2.08 (1.95 – 2.23), 3.23 (2.39 – 4.37)) (35). The effect of maternal obesity on offspring birth weight might partly be mediated by higher placental weight, which has been proposed in obese pregnancies along with vascular dysfunction, inflammation and alteration in transport and mitochondrial activity (22). Due to larger babies and a narrower birth canal, increasing the risk of dystocia, obese women are more likely to deliver their offspring by cesarean section (C-section) (OR (95% CI) for women with preconception BMI ≥ 30.0 kg/m^2 and ≥ 35.0 kg/m^2: 2.05 (1.86 – 2.27) and 2.89 (2.28 – 3.79), respectively) (36).

1.2.2 Maternal postpartum outcomes

Postpartum outcomes related to maternal obesity include lower breastfeeding rates, more weight retention and adverse effects on metabolic and cardiovascular health (21). Maternal postpartum weight retention i.e. interpregnancy weight gain, showed a dose-response relationship with obesity-related pregnancy complications for subsequent pregnancies. Weight retention, which is more common in preconception obese women, increases the risk for comorbidities associated with obesity even at modest increases of 2-3 BMI units (21). Further, obesity-related pregnancy complications were associated with adverse long-term metabolic and cardiovascular outcomes: obese women, who developed a GDM during pregnancy were at an increased risk for type 2 diabetes (T2D) (RR 2.85 (95% CI) 1.69 – 3.69) in later life (37), whereas those with pre-eclampsia have a life-long risk for cardiovascular diseases including a four times increased risk for hypertension, and two times increased risk for ischemic heart diseases and stroke (38). Obese women are 13% less likely to initiate breastfeeding and 20%

less likely to breastfeed after six months (39), even though breastfeeding has been shown to reduce risk of obesity related comorbidities such as diabetes, hypertension and high cholesterol (40). Lack of initiation has been related to mechanical factors, e.g. difficulty finding a good breastfeeding position and delayed onset of lactogenesis II, while impacting hormonal imbalances, psychosocial factors, and mammary hypoplasia are related to shorter durations of breastfeeding (40).

1.2.3 Offspring postnatal outcomes

Adverse offspring outcomes associated with maternal obesity are mostly related to offspring overweight and obesity, and its associated cardiovascular and metabolic diseases (22-24).

Supposedly, maternal obesity (BMI $\geq$ 30.0 kg/m^2) explains up to 21.6% of the childhood overweight/obesity prevalence (based on WHO cut offs), while maternal overweight (BMI $\geq$ 25.0 kg/m^2) covers a total of 41.7% (41). Evidence suggests that maternal obesity is among the most important factors for childhood overweight/obesity (17), which in turn is a predictor for obesity in adulthood (18). The risk for childhood overweight/obesity associated with maternal preconception obesity increases progressively with obesity classes and across the full range of BMI (41, 42). Offspring of preconception obese mothers have a 2.4 to 4.5 times higher risk for childhood overweight/obesity, which increases with offspring age (OR (95%) in early (2-5 years) and late childhood/adolescence (10-18 years): 2.43 (95% CI: 2.24 - 2.64]) and OR 4.47 (3.99 - 5.23) (41, 42). As suggested by findings from large birth cohorts, associations with maternal BMI and offspring BMI are still apparent in mid (32 years) and late (62 years) adulthood (43, 44). Also higher total and relative body and abdominal fat mass were reported in 5-6-year-old offspring of obese mothers (45-47).

Irrespective of maternal BMI, the greatest growth acceleration related to sustained obesity occurs between two and six years of age (48), pointing to early childhood as a critical phase for the development of later obesity risk and a possible risk modifier for later obesity. High birth weight, macrosomia and LGA birth weight, which are all associated with maternal obesity (35), are risk factors for childhood overweight/overweight (49). Further, early BMI trajectories of offspring of obese mothers are twice as likely (RR (95% CI): 1.96 (1.36–2.83) to be characterized by a rapid weight gain in infancy and a high, stable BMI until the age of four years resulting in childhood obesity (BMI $\geq$95th percentile according to the Center for Disease Control and Prevention (CDC) growth charts) at this age (50). Rapid infant weight gain (a change in weight z-score > 0.67) is an independent risk factor for obesity in child and adulthood (51). Further, maternal preconception BMI is a strong, independent risk factor for very early (<3.5 years) and early (<5 years) adiposity rebound (52-55), which in turn is associated with risk of later obesity (56-58). The term “adiposity rebound” refers to the age period during

childhood, generally about 6 years of age, that corresponds to the nadir of the BMI curve when BMI starts to raise again after a rise in infancy and subsequent decline (56-58).

In the context of maternal preconception obesity and offspring overweight/obesity as well as adverse anthropometrical outcomes such as higher waist circumference and fat mass in child and early adulthood, both GDM and excessive GWG are often discussed as independent and additive prenatal risk factors (41, 59-64). Interestingly, studies showed that GDM only exhibits adverse effects in combination with maternal obesity (61-63). The effect attenuates greatly when adjusting for maternal BMI (60, 64). Further, the effect of an excessive GWG in obese pregnancies was rather small, which highlights the importance of maternal obesity as a main influencing factor in relation to offspring obesity related outcomes (41).

Studies investigating modifiable risk factors for childhood overweight/obesity suggest that the risk for childhood obesity increases with the quantity of risk factors the offspring is exposed to during the first 1000 days of life (65, 66). The exposure to several risk factors occurs frequently in obese mothers as maternal obesity increases the odds of several pregnancy complications. The risk for childhood overweight/obesity increases up to 11.1 times when exposed to 4-5 factors, including maternal and paternal BMI, excessive GWG, GDM, breastfeeding duration and introduction of solid food. Noteworthy, even when considering postnatal factors, maternal obesity remains the strongest risk factor (65, 66).

Maternal obesity was further found to be associated with insulin resistance, higher blood pressure and adverse lipid profile, i.e. low high-density lipoprotein (HDL) cholesterol and high triglyceride (TG) concentration and inflammatory markers in childhood (47, 59, 67, 68) and adulthood (43), though some suggest that these associations were mostly mediated by offspring BMI. In addition, a 3.5 times higher risk of T2D in the offspring diagnosed between the ages ten to 61 was attributed to maternal obesity (69). Further, maternal obesity was associated with early pubertal timing in girls and boys (70, 71). Other adverse outcomes include increased risk for asthma and adverse neurocognitive and behavioral development, including poorer cognitive outcomes, autism spectrum disorder, attention deficit hyperactivity disorder, increased susceptibility to immune and infectious diseases. However, these outcomes are not as extensively studied, and identified risks are moderate or inconsistent (24). Though evidence clearly suggests that maternal obesity may be a significant risk factor for offspring obesity and its comorbidities, underlying mechanism remain uncertain.

1.3 Fetal programming in obese pregnancies

Genetic predisposition, environmental influences, lifestyle and socio-demographic factors are known risk factors for obesity (22-24, 41). In addition, the notion of direct effects of an altered

intra-uterine milieu the so-called fetal programming has become more prominent as potential cause for the vicious cycle of obesity and its interrelated risk factors (22-24, 41) (Figure 1).

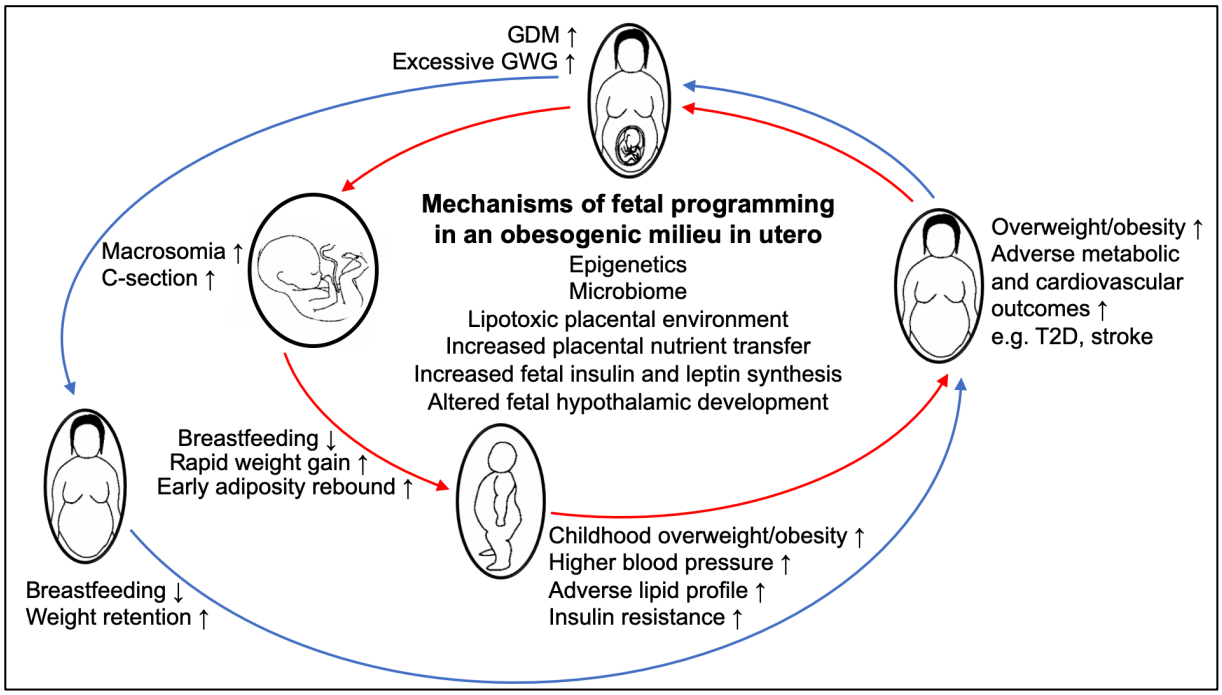

Figure 1: Fetal programming and the vicious cycle of obesity. Besides genetic predisposition, environmental influences, lifestyle and socio-demographic factors, fetal programming is discussed as causal for obesity via several mechanisms (22-24, 41). Well established risk factors for adverse metabolic outcomes that are altered in response to maternal obesity are provided along with corresponding outcomes. GDM, gestational diabetes mellitus; GWG, gestational weight gain; T2D, type 2 diabetes.*Source: Figure adapted from* (72).

1.3.1 Definition and indications

Fetal or intrauterine programming was first described in relation to maternal undernutrition and low birth weights resulting in offspring obesity (73) and cardiovascular and metabolic diseases later in life (74, 75). Adverse outcomes occurred supposedly due to a mismatch between prenatal adaptations to poor fetal nutrition in utero, in order to maximize survival and abundance of food postnatally (76). This concept is now known as the “Developmental Origins of health and Diseases” hypothesis. It states that fetal undernutrition during critical phases of developmental plasticity in early life, i.e. in utero and infancy, can permanently change structure, physiology and metabolism and predispose the individual to cardiovascular diseases later in life (77). This concept was then also investigated in relation to an adipogenic environment in utero. Indications herefore were U-shaped connections between birth weight and the cardiometabolic risk in later life, i.e. association of reduced and increased birth weight with adverse outcome (Figure 2) (78, 79), along with the increasing prevalence rates and transgenerational transmission of obesity.

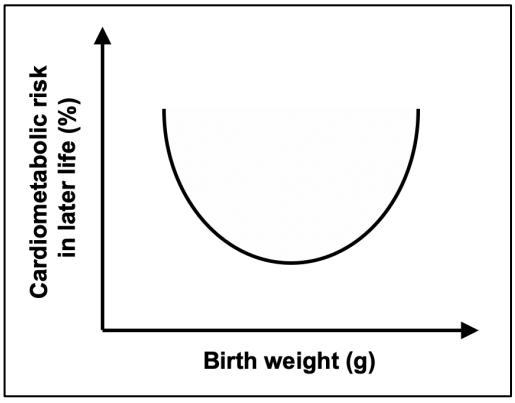

Figure 2: U-shaped relationship between birth weight and cardiometabolic risk in later life.*Source: Figure adapted from* (78).

Corresponding to the Developmental Origins of Health and Diseases hypothesis, the fetal overnutrition hypothesis proposes that fetal exposure to an excess supply of nutrients during critical phases of organ and tissue development can equally induce permanent changes, which might make individuals more susceptible to adverse health outcomes including obesity in later life (22, 76, 80).

Indications for an intrauterine programming in offspring of obese are provided by sibling comparison studies as they naturally minimize confounding factors such as genetic predisposition and environmental factors (81, 82). Among mothers who had substantial weight loss in between pregnancies due to bariatric surgery, the risk for offspring preobesity, obesity, and adverse cardiometabolic risk factors was higher in the sibling conceived before bariatric surgery, i.e. before weight loss (81, 82). Investigation of the epigenetic profile of offspring born before and after bariatric surgery of a morbidly obese mother showed different methylation patterns further supporting this hypothesis (83). In addition, stronger effects of maternal than paternal BMI on offspring risk for overweight support the notion of an influence of the intrauterine milieu (47, 84, 85).

1.3.2 Underlying mechanisms

Maternal preconception obesity and associated factors such as poor nutritional status, altered body fat distribution, insulin and glucose metabolism and low-grade inflammation may affect the intrauterine environment and induce fetal programming via epigenetic mechanisms, placental and fetal adaptions (86).

Epigenetic modifications have been proposed as an important underlying mechanism (24, 76). Via epigenetic mechanisms such as DNA methylation histone modification and microRNA variation, the gene expression is modified altering the phenotype but not the genotype (76). Methylation of DNA, which occurs at the fifth position of cytosine in CpG dinucleotides, inhibits

gene expression (87). As the methylation of DNA is linked to dietary supply of methionine (Met) via the one-carbon metabolism, early malnutrition could alter epigenetic mechanisms (88). Though findings are not consistent, there is evidence that maternal BMI is associated with DNA methylation of genes involved in infectious, inflammatory and lipid metabolism in umbilical cord blood (UCB) (89). Further, methylation of human UCB associated with maternal preconception obesity, is linked to offspring adiposity and BMI (90) in a sex-specific manner (91). Interestingly, Sharp et al. not only investigated associations with maternal but also paternal BMI, showing stronger effects of maternal BMI, which further supports the hypothesis of intrauterine effects (90). Also, methylation of genes involved in immunological and inflammatory pathways differed in peripheral blood of offspring conceived before and after maternal bariatric surgery (92).

Other mechanisms contributing to fetal programming might be related to placental changes induced by maternal obesity including a lipotoxic placental environment. Lipid accumulation in placentas of obese mothers lead to the activation of signaling cascades, which promote the development of proinflammatory cytokines and oxidative stress, which in turn could negatively affect fetal growth (93).

Further, animal studies showed an increased transplacental transport of macronutrients such as glucose from maternal to fetal circulation might accelerate the fetal pancreatic development, β-cell dysfunction and fetal hyperinsulinemia (94). Along with higher concentrations of insulin and connecting peptide (C-peptide) (95), higher concentrations of leptin have been reported in human UCB to be related to higher maternal BMI (96). Increased leptin and insulin concentration may play a major role in programming of the hypothalamus, which is associated with altered appetite control leading to hyperphagia and alterations in satiety mechanisms as evident from animal models (80, 94). Further offspring adipocyte morphology and metabolism might be negatively affected by maternal obesity, possibly contributing to obesity and insulin resistance in later life (97).

Another mechanism proposed to confer obesity from mother to offspring is linked to the microbiome: the compositional structure of the offspring's gut microbiota might be altered by maternal overweight through vertical transfer of microbiota and/or their metabolites in utero, at delivery and breastfeeding (98).

1.4 Metabolites in umbilical cord blood

The UCB metabolome mirrors the intrauterine milieu (99, 100). UCB is fetal blood, that flows oxygenized and nutrient-rich from placenta to the fetus in the umbilical vein and transports

waste products and deoxygenized blood via the two umbilical arteries back to the placenta (101). UCB contains all elements of human adult whole blood i.e. plasma, platelets, red and white blood cells (102). However, its metabolome comprises a mixture of metabolites from maternal circulation and metabolism, placental metabolism, and metabolites from fetal metabolism and synthesis (99, 100). Thus, a UCB metabolite profile indicates alterations in nutrient supply and fetal metabolism.

The growing fetus mainly relies on nutrients from exogenous sources (103, 104). To enter fetal circulation, nutrients have to cross the syncytiotrophoblast and the fetal capillary epithelium (Figure 3). The syncytiotrophoplast is the transporting epithelium of the human placenta which lines the villi and has two polarized membranes, the microvillous plasma membrane and the basal membrane. The microvillous plasma membrane is oriented towards the maternal circulation and the basal membrane towards the fetal circulation. Both membranes express nutrient transporters for transplacental transport. Expression is regulated by fetal, maternal and placental signals. As the fetal capillary epithelium is permeable for molecules such as amino acids, the syncytiotrophoblast presents a barrier and the rate-limiting step for the transport of nutrients from maternal to fetal circulation. Other factors influencing the transplacental transport are nutrient availability, placental metabolism, uteroplacental and umbilical flows, and area for exchange (103, 104).

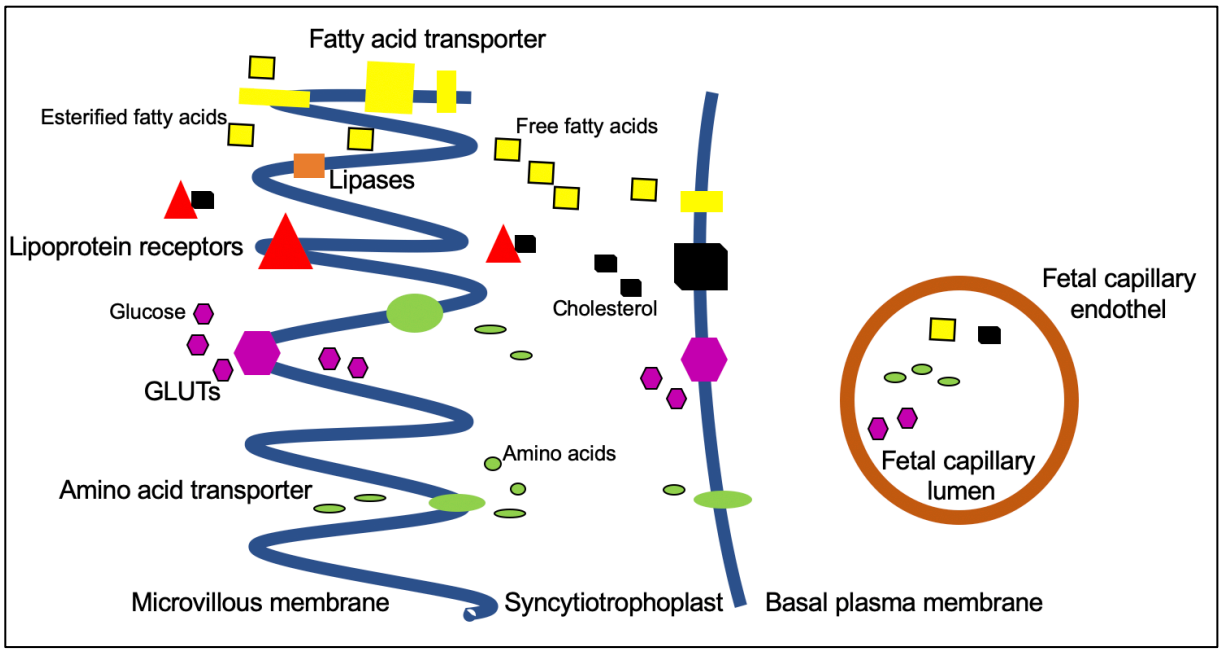

Figure 3: Transplacental transport. Established transporters located at the microvillous membrane and the basal plasma membrane that mediate the transport of nutrients via the syncytiothrophoblast are depicted. These transporters include FATP, FAT and FABPpm for transport of fatty acids which are transported in their free form after hydrolysis of esterified fatty acids by lipases, GLUT for transport of glucose, various amino acid transporters for transport of amino acids (103, 104). Cholesterol is transported via the basal plasma membrane after uptake and breakdown of lipoproteins in the placenta

(104). FATP, fatty acid transport proteins; FAT, fatty acid translocase; FABPpm, plasma membrane fatty acid binding protein; GLUT, glucose transporter.*Source: Figure adapted from* (103).

Maternal obesity is associated with overnutrition causing adverse alterations in glucose and lipid metabolism, leptin and adiponectin concentration, and secretion of growth factors and inflammatory cytokines (105). All of these factors can modulate placental function and metabolism increasing nutrient transport to the fetus and promoting fetal overgrowth.

1.4.1 Amino acids

The fetus mainly depends on amino acids from maternal circulation (104). Amino acids are essential for fetal protein synthesis, oxidative metabolism and biosynthesis (106). Higher amino acid concentrations in the fetal than in the maternal circulation (107) indicate an active transport mediated by one of the more than 20 amino acid transporters (accumulative transporters, exchangers, and facilitated diffusion)(108). Each transport can transfer several amino acids and each amino acid can be transported by several transport systems (106). System A, a sodium dependent accumulative transporter for uptake of small neutral amino acids (SNAT) and system L, a sodium independent exchanger for large neutral amino acid transport (LAT) exchanging non-essential for essential, branched chain or aromatic, amino acids are the best studied transport mechanisms (103, 104, 106). Both systems are expressed at both membranes with higher expression at the microvillous plasma membrane (103, 104, 106).

Apart from the amino acids that are transferred from maternal to fetal circulation via the placenta, the fetus also relies on placental metabolism to meet its amino acid requirements (109). The placenta is a metabolically active organ, which does not only regulate transfer but also oxidizes, synthesizes and interconverts nutrients, which can then be transferred to the fetal circulation for adequate supply. For example, an interorgan cycling has been suggested for the amino acids serine (Ser) and glycine (Gly): Ser from maternal and fetal circulation is converted to Gly in the placenta and transferred to the fetal circulation, where the fetal liver can reconvert Gly to Ser (109).

1.4.2 Phospholipids

Due to their amphiphilic character, phospholipids such as phosphatidylcholines (PC), lysophosphatidylcholines (LysoPC) and sphingomyelins (SM) are an important structural element in cellular membranes (110).

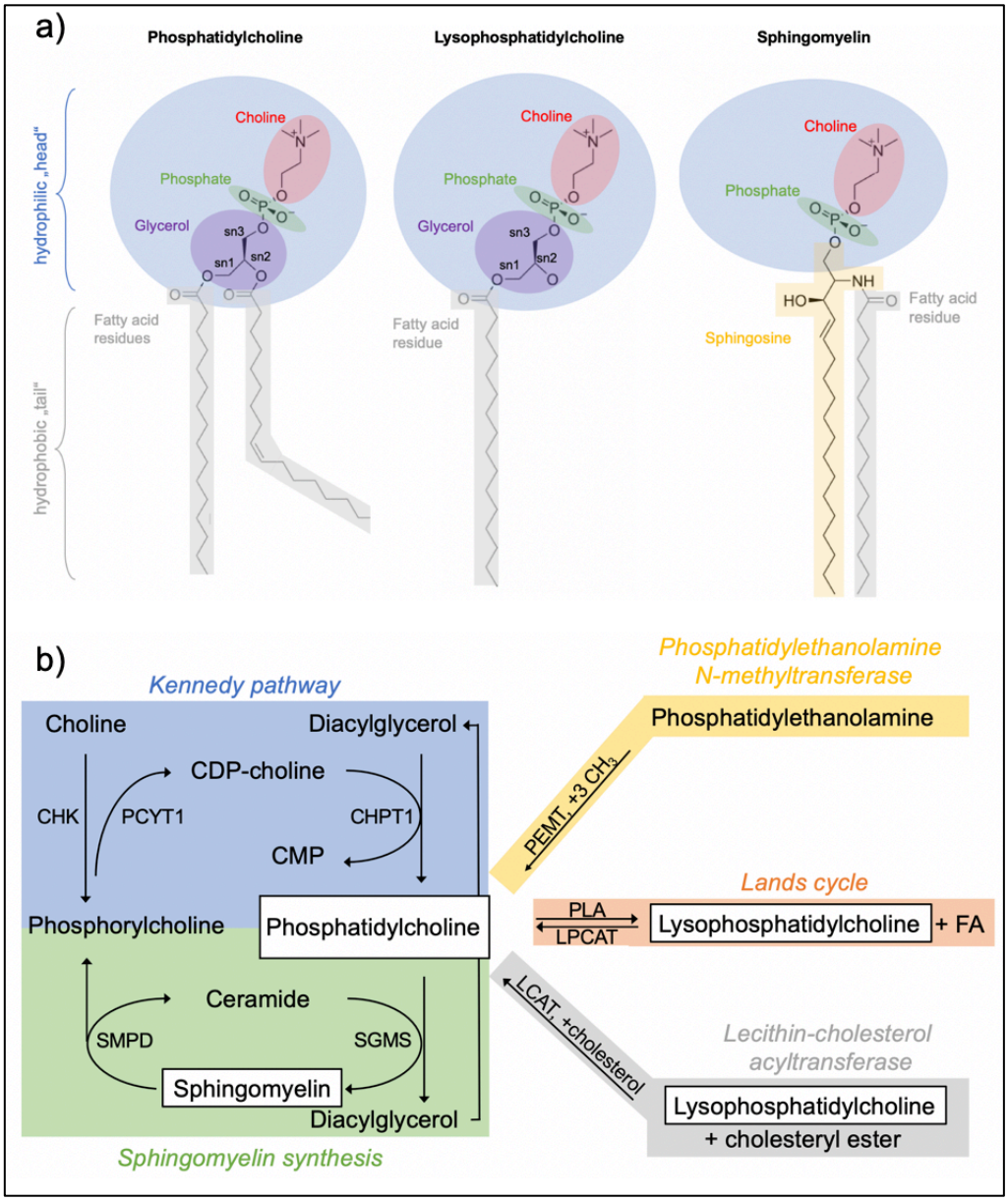

Figure 4: Amphiphilic structure and biosynthesis of analyzed phospholipids. a) Phosphatidylcholine and lysophosphatidylcholine contain choline, a phosphate group and a glycerol backbone in the hydrophilic head group and two or one fatty acid residue of different length and saturation as hydrophobic tail, respectively (110, 111). Sphingomyelins equally contain choline and a phosphate group in the hydrophilic head group, whereas their hydrophobic tail comprises ceramide, i.e. sphingosine and a fatty acid residue (110). b) Phosphatidylcholine is synthesized via the Kennedy pathway in the endoplasmatic reticulum of all nucleated cells and from the subsequent methylation of phosphatidylethanolamine in hepatocytes (110, 111). The reversible de-acetylation of phosphatidylcholine within the Lands cycle results in lysophosphatidylcholine (111). Lysophosphatidylcholine also stems from the esterification of cholesterol and a fatty acid residue derived from phosphatidylcholine (112). Sphingomyelin is made from the reaction of phosphatidylcholine and ceramide (110). CDP, cytidine 5'-diphosphocholine; CHK, choline kinase; CMP, cytidine monophosphate; CHPT1, choline phosphotransferase 1; PCYT1, choline-phosphate cytidylyltransferase; FA, fatty acid; LCAT, lecithin-cholesterol acyltransferase; LPCAT, lysophosphatidylcholine acyltransferase; PEMT, phosphatidylethanolamine N-methyltransferase; PLA, phospholipase A; SMPD, sphingomyelin phosphodiesterase; SGMS, sphingomyelin synthase-1. *Source: Figure adapted from* (113) (114).

PCs, the most abundant phospholipids species, consist of a hydrophilic choline and phosphate head group, a glycerol backbone and two fatty acid residues (Figure 4a) (110). PCs usually

contain saturated fatty acids at their sn1 position, and saturated or unsaturated fatty acids at the sn2 position (115). LysoPCs have the same structure but contain one instead of two fatty acid residues (111). In contrast, SMs consist of a choline and phosphate head group, sphingosine and a fatty acid residue (110).

PCs are synthesized de novo from choline and diacylglycerol in the endoplasmatic reticulum of all nucleated mammalian via the Kennedy pathway (Figure 4b) (110, 111). In hepatocytes, PCs are also synthesized from subsequent methylation of phosphatidylethanolamine (110, 111). Via the Land's cycle, PCs can then be converted into LysoPCs and vice versa (111). Hydrolysis of PC resulting in LysoPC occurs via phospholipases A_2 at the sn2 position (111), though a phospholipase, i.e. phospholipase A_1, which hydrolyzes at the sn1 position, exists (116). LysoPC is further formed when cholesterol is esterified with a fatty acid residue from PC (112). SMs are synthesized from ceramide, i.e. sphingosine and a fatty acid residue, and PCs in the golgi apparatus via the sphingomyelin synthase (SMS), mainly the SMS1 (110).

Phospholipids themselves cannot be transferred via the membranes of the syncytiotrophoblast (104), thus, the fetus presumably incorporates free fatty acids from endogenous synthesis or exogenous sources into phospholipids as suggested by studies from the 1970's (117). Though the fetus can synthesize some saturated and monosaturated fatty acids from glucose, the fetus relies on maternal supply of the essential omega-3 and omega-6 fatty acids and long-chain polyunsaturated FAs (118). Fatty acids can only be transported across the placenta in its free from. Thus, fatty acids incorporated in maternal lipoproteins e.g. triglycerides (TG) and phospholipids, are hydrolyzed by lipoprotein lipase (119) and endothelial lipase (120), respectively, in the microvillous plasma membrane before they enter the placental cytosol via fatty acid transport proteins (FATP), fatty acid translocase (FAT), and plasma membrane fatty acid binding protein (FABPpm) or passive diffusion (118).

1.4.3 Bile acids

Bile acids are necessary for digestion and elimination of waste products (121). Bile acids are divided into primary and secondary (Figure 5a). The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized in the liver from cholesterol via the classical and alternative pathway, respectively. The classical pathway is the dominant pathway in adults and consists of numerous separate steps including cytochrome P450 enzymes. In alternative pathway, cholesterol is first transformed extrahepatically before it enters the liver and is further modified. The primary bile acids can be converted into secondary bile acids by the intestinal microbiota. The primary bile acid CA is converted to the secondary bile acid deoxycholic acid (DCA), while the primary bile acid CDCA is converted into the secondary bile acids lithocholic

acid (LCA) and ursodeoxycholic acid (UDCA). For higher hydrophilicity and lower cytotoxicity, bile acids are conjugated with Gly or taurine (Figure 5) (121).

Most bile acids are conjugated into bile salts in the liver before they are excreted into the small intestine upon food intake (121, 122). The majority of bile salts is then actively reabsorbed in the distal ileum and transported back to the liver via the portal vein. A small fraction of bile salts is deconjugated and passively reabsorbed, or, in case of primary bile acids, can further be dehydroxylated by intestinal microbiota to form new secondary bile acids. Secondary bile acids can then be reabsorbed in the colon or excreted via feces (121, 122).

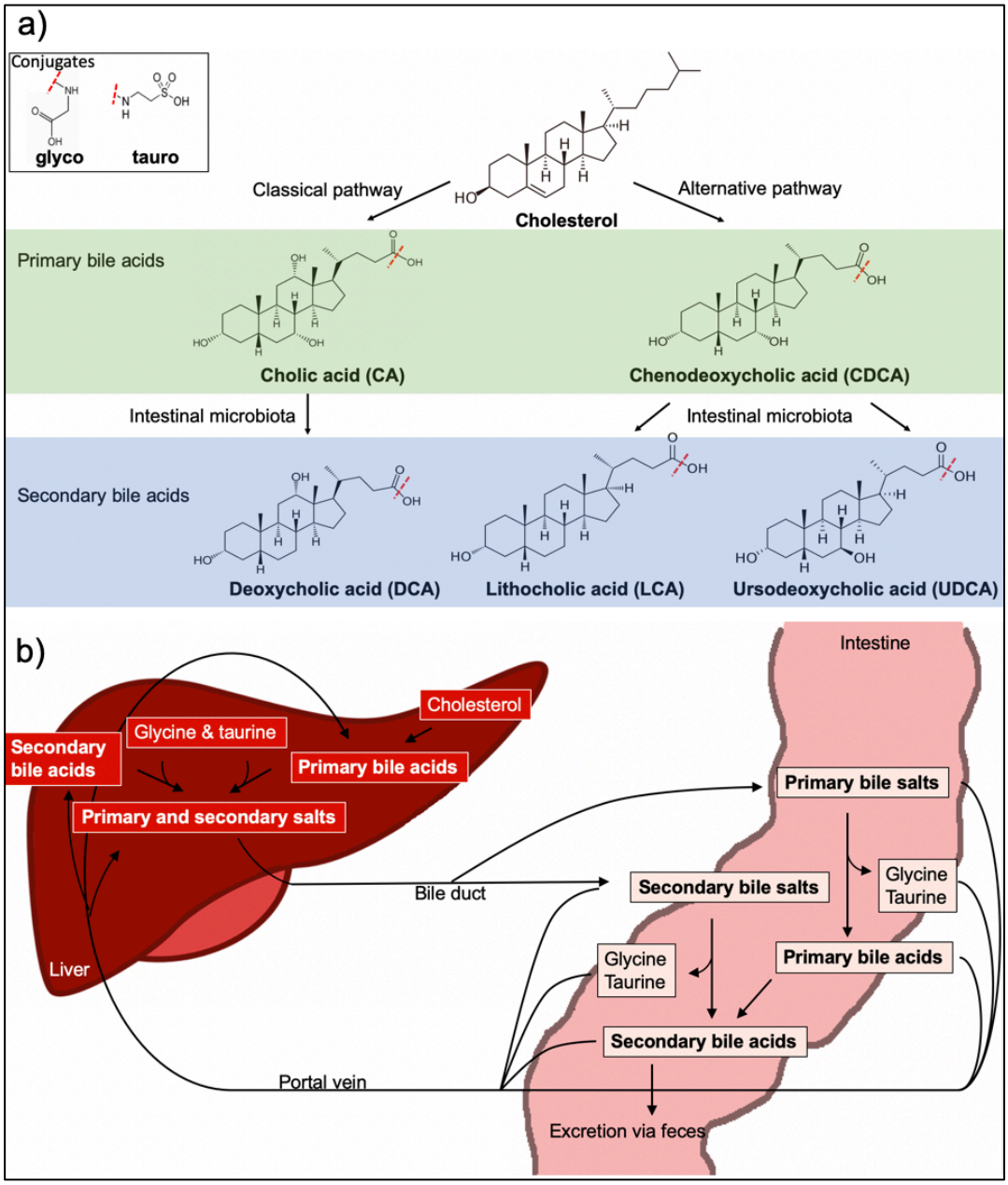

Figure 5: Structure, synthesis and metabolism of analyzed bile acids. a) Primary bile acids are synthesized by several enzymatic steps from cholesterol via the classical and alternative pathway in the liver (121). Primary bile acids can further be converted to secondary bile acids by the intestinal

microbiota. Bile acid can be conjugated with glycine and taurine (121). b) Primary and secondary bile salts are excreted into the intestine via the bile duct (121, 122). The majority is reabsorbed without transfomation. A small fraction is deconjugated and in case of primary bile acids dehydroxylated to form secondary bile acids by gut microbiota before they are reabsorbed and transported back to the liver via the portal vein or exctred via feces (121, 122). *Source: Figure adapted from* (123-129).

The fetal capability for endogenous cholesterol synthesis increases over the course of gestation (130). However, the microvillous plasma membrane also expresses low-density lipoprotein receptor (LDLR), scavenger receptor class B type I (SRBI) and very low-density lipoprotein receptor (VLDLR) for the uptake of low-density lipoprotein (LDL), HDL and very low-density lipoprotein (VLDL) cholesterol, which internalize and degrade lipoproteins and free cholesterol for transfer across the basal membrane. They transport cholesterol via ATP binding cassette (ABC) transporters subfamily A member 1 and subfamily G member 1 to the fetus (104). The fetus synthesizes bile acids as of the 12th week of gestation (131), mainly via the alternative pathway (132). In contrast to adults, taurine-conjugates present the majority of fetal conjugates (133). As prenatal bacterial colonization has been reported despite the prevalent notion of a sterile intrauterine environment (134), secondary bile acids might also be synthesized by the fetus. Due to the immature hepatobiliary and renal systems, the fetus relies on the placenta for elimination of waste products and toxic compounds. Members of the organo anion (OAT) and ABC transporter are discussed to transfer fetal bile acids to the maternal circulation for excretion. There is evidence that bile acids are also transferred from the maternal to the fetal circulation, however, this is not clearly proven (121).

1.4.4 Acyl-carnitines

Acyl-carnitines are the esters of activated fatty acids i.e. acyl-CoA (coenzyme A) and L-carnitine (Figure 6) (135). The plasma acyl-carnitine concentration reflects the tissue pool of acyl-CoA (136).

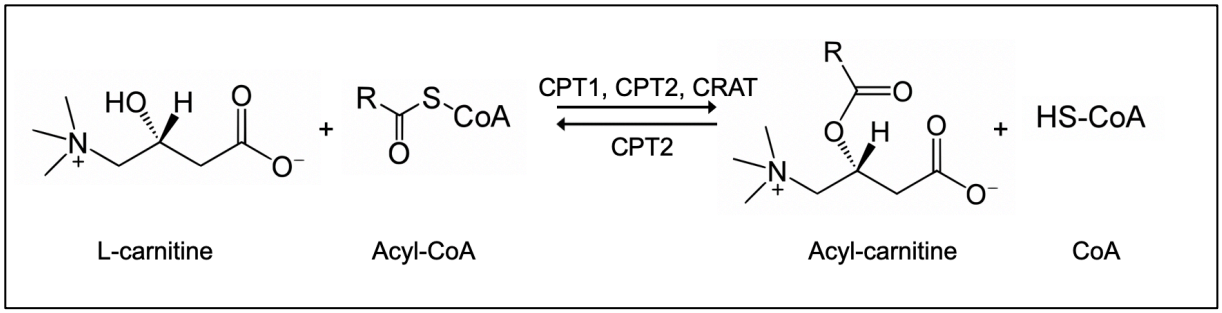

Figure 6: Structure and mitochondrial synthesis of acyl-carnitines(133). CoA, coenzyme A; CPT1, carnitine palmitoyltransferase I; CPT2, carnitine palmitoyltransferase II; CRAT, carnitine O-acetyltransferase. *Source: Figure based on* (137).

Acyl-carnitines are known as byproducts of the BCAA catabolism (138) and for their role in β-oxidation (135). For mitochondrial β-oxidation, formation of acyl-carnitines via carnitine

palmitoyltransferase I (CPT1) is essential to transport fatty acids from the cytosol into the mitochondria via the mitochondrial carnitine/acylcarnitine carrier protein (solute carrier family 25 member 20, SLC25A20). In the mitochondria carnitine palmitoyltransferase II (CPT2) reverses this reaction. CPT2 can also catalyze the formation of medium- and long-chain acyl-carnitines to enable the transport of accumulating β-oxidation products into the cytosol (135). This reaction is also performed by carnitine O-acetyltransferase (CRAT) with a preference for short acyl-CoAs in order to maintain the mitochondrial acyl-CoA/CoA pool (139). These acyl-carnitines can then be transported via the reverse action of SLC25A20 (135). It remains unknown how acyl-carnitines then cross the plasma membrane to enter circulation (140). The organic cation/carnitine transporter 2 (solute carrier family 22 member 5, SLC22A5), which transports carnitine into the cytosol, was hypothesized to also export acyl-carnitines into the circulation (141). Instead, an involvement of SLC25A20 and CPT2 was suggested by the experimental results using human fibroblasts (141). Apart from its activity in mitochondria, CRAT along with carnitine O-octanoyltransferase (CROT) exhibits its enzymatic activity in the peroxisome, where very long chain acyl-CoAs are oxidized prior to complete oxidation in the mitochondrium (139). Whether acyl-carnitines are transferred to the fetal circulation from maternal circulation or placenta can only be presumed from early studies which demonstrate the transfer of the short-chain acetylcarnitine in contrast to the long-chain palmitoylcarnitine (142). Along with the transport of free fatty acids via FATP, FAT and FABPpm from maternal to fetal circulation (118), transport of L-carnitine across the microvillous membrane via the SLC22A5 has been reported (143). However, it remains unknown how L-carnitine is transported across the basal plasma membrane.

1.5 Metabolite profiling in UCB in relation to maternal obesity and weight outcome

Metabolite profiling enables the investigation of metabolite concentrations and metabolic pathways in biomaterials such as blood and tissue. Thus far, most studies in this area have used targeted approaches investigating amino acids, acyl-carnitines, phospholipids, and non-esterified fatty acids (99, 100, 144-147).

Studies analyzing the impact of maternal obesity on UCB metabolite profiles as well as studies on the relationship between UCB metabolite profiles and adverse offspring weight outcome are limited. Few studies have investigated the UCB metabolite profile in relation to the maternal preconception BMI (99, 144). Promising evidence on the effects of the maternal BMI, measured at mid-pregnancy, on the UCB metabolite profile across its full range was reported based on UCB samples from 1600 offspring of the multicentric Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (99). Authors presented a metabolic signature in UCB,

including branched-chain amino acids (BCAA) and their metabolic byproducts to be associated with both, maternal BMI and maternal insulin resistance (99). Similarly the Prospective Randomized Open Blinded End-Point (PROEBE) study found explorative trends for associations between maternal preconception BMI and UCB metabolites including BCAAs upon the targeted investigation of 111 UCB samples (144).

However, basically nothing is known on the effect of an obesogenic environment on the UCB metabolite profile despite the high prevalence rates of overweight at conception. So far only a small case-control study (n = 28 cases vs. n = 29 controls) used a coupled targeted and untargeted approach and proposed a set of 29 metabolites including organic acids, phospholipids, acyl-carnitines and amino acids as potential early-life biomarkers that may indicate the effect of maternal obesity in utero (148). However, models lacked adjustment for confounding variables when analyzing UCB metabolites such as GDM, GWG, gestational age, offspring sex, mode of delivery, and birth weight as used by others (99, 100, 144, 147). Further, only few of the proposed UCB metabolites were individually associated with maternal obesity (148). As they would not have remained significant after adjustment for multiple testing, results might rather be regarded as explorative trends.

Though preschool age is a critical time in weight development as the greatest growth acceleration related to sustained obesity occurs between two and six years (48), evidence regarding the relationship between metabolic alterations in UCB and offspring weight outcome is mostly limited to birth weight. Such associations were identified, independent (99) and irrespective (149) of maternal BMI, and in offspring of normal weight (100, 146, 150) and obese (147) mothers. Metabolites associated with higher birth weight included medium-chain acyl-carnitines (99, 150), BCAA metabolites (149), and LysoPCs (100, 146, 147, 150). Significant findings on the relationship between UCB metabolites and young infant weight outcome beyond birth were only reported by the UK Pregnancies Better Eating and Activity Trial (UPBEAT) investigating 344 mother-child pairs, which found principal components of PCs in UCB of offspring of obese mothers not only to be associated with birth weight but also with rapid weight gain and weight at six months.

Further, some studies provided associations of rather explorative nature between the UCB metabolites and later weight outcome (100, 145, 151, 152), however, still worth mentioning as not much else is known. They were characterized by a lack of adjustment for multiple testing and other limitations such as small cohorts and clinical heterogeneity between study groups. Findings of one study included associations of a set of metabolites, such as one-carbon metabolites, with offspring rapid postnatal weight gain resulting in mid-childhood overweight

(151). Another study found UCB medium- and long- chain fatty acids to be associated with childhood obesity between three and five years (152). An inverse relationships was proposed between LysoPCs and weight of offspring of normal weight mothers at two and 18 years (100). Moreover, differences in UCB metabolites were suggested to be associated with weight at one year in offspring of normal weight and obese mothers (145).

Even though the current literature provides some useful insights into the impact of maternal preconception BMI on UCB metabolites, significant associations describing the influence of maternal preconception obesity on UCB metabolites are lacking. Further, the extent of maternal obesity has not been examined in this context. Also, no information is available on how and if additional adverse maternal factors associated with maternal obesity, such as maternal hyperglycemia, might impact the UCB metabolome. Even less is clear about the relationship between offspring metabolites at birth and offspring weight outcome beyond infancy. It remains unknown, if, and if yes, which metabolites are associated with adverse childhood weight outcome, and whether or not these metabolites might depend on maternal preconception BMI.

1.6 Hypothesis and research aims

Accumulating evidence suggests that maternal obesity is among the most important prenatal risk factors for childhood overweight/obesity (17), which predisposes the offspring for overweight/obesity throughout the life course (18). However, the full scope of underlying mechanisms relating early adipogenic exposure to child weight outcomes remains unknown. One hypothesis is that in offspring of obese mothers, the origins of overweight/obesity are induced by mechanisms related to fetal programming, possibly with a maternal BMI “dose-dependent” effect (22-24, 41). Such programming permanently alters the offspring’s metabolism during critical periods of fetal development (22, 76, 80). As the UCB metabolome reflects the intrauterine milieu (99, 100), metabolites in UCB might function as a surrogate marker for metabolic programming. Such markers might link maternal preconception obesity to adverse metabolic outcomes in the offspring and might potentially allow for identification of children at risk for the development of overweight as early as at birth.

Using a targeted liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) approach, to provide a basis for the main analysis of this thesis, we first aimed to (1) determine differences in amino acids and acyl-carnitines in UCB of offspring of normal weight, healthy women and metabolically healthy, female adult blood. For the main analysis, we aimed to (2) identify alterations in UCB metabolite profiles including specific metabolites and metabolic pathways in offspring of obese and severely obese mothers compared to offspring of normal weight mothers and to confirm alterations in the metabolism by analyzing relative mRNA

expressions of selected genes of enzymes involved in altered pathways. Furthermore, we aimed to (3) identify alterations in UCB metabolites in offspring of obese mothers with additional late-pregnancy dysglycemia compared to obese mothers without late-pregnancy dysglycemia. Finally, we aimed to (4) determine whether metabolic alterations in UCB are associated with adverse longitudinal BMI development and BMI outcome at different ages during preschool life of offspring of normal weight as compared to obese mothers.

2 Material and Methods

2.1 Study design

2.1.1 Mother-child cohort PEACHES

The prospective mother-child cohort PEACHES comprises 1707 offspring and their mothers recruited during pregnancy to investigate the short- and long-term effect of preconception maternal obesity and GDM on offspring and maternal health outcomes (153-155). Figure 7 shows the longitudinal design of the PEACHES study. The study was approved by the local ethics committee of the Ludwig-Maximilians-Universität München, Munich, Germany (protocol number: 165–10). Written informed consent was obtained from all participants.

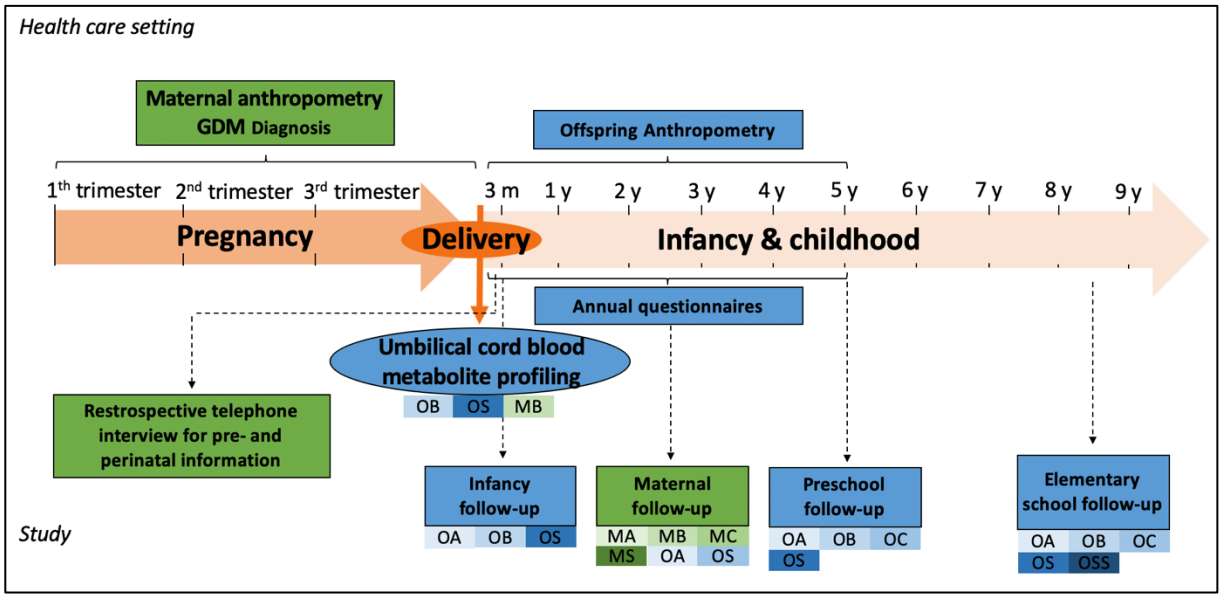

Figure 7: Study design of the mother-child cohort PEACHES. m: months; MA: Maternal anthropometry; MB: Maternal blood sample; MC: Maternal cardiovascular measurements; MS: Maternal buccal smear; OA: Offspring anthropometry; OB: Offspring blood sample; OC: Offspring cardiovascular measurements; OS: Offspring buccal smear; OSS: Offspring stool sample; y: years.

Preconception obese and normal weight pregnant women with and without GDM were recruited from 2010 until 2015, 4–6 week's prior to their due date by their midwives or obstetricians at their maternity clinic upon the first visit to maternity wards or midwifery. Recruitment was conducted in cooperation with maternity clinics of the Ludwig-Maximilians Universität München and other maternity clinics in the Munich area and Bavaria, the Heinrich Heine University Düsseldorf and parts of northern Germany (Table 3).

Table 3: Participating maternity hospitals

Krankenhaus Agatharied	Krankenhaus Landshut-Achdorf
Kreiskliniken Altötting-Burghausen	Marienhospital Osnabrück
Klinikum Augsburg	Klinikum Memmingen
Amper Kliniken AG Dachau	Klinikum München-Pasing

Klinikum Dritter Orden, München	Klinikum Neuperlach, München
Universitätsklinikum Düsseldorf	Klinikum Rechts der Isar, München
Frauenklinik Maistraße	Klinikum Rosenheim
Klinikum Fürstenfeldbruck	Rotkreuzklinikum München
Klinikum Garmisch-Partenkirchen	Klinikum Schwabing
Klinikum Großhadern	St. Elisabeth Krankenhaus Jülich
Klinikum Harlaching	Klinikum Traunstein
Heidekreis-Klinikum Walsrode	Klinikum Weilheim
Josefinum in Augsburg	

Maternal age of at least 18 years, written informed consent, preconception BMI <25.0 or ≥30.0 kg/m^2, singleton, term pregnancy and absence of pre-existing type 1 diabetes or T2D or other chronic diseases were required for inclusion.

At delivery, biomaterials from mother and offspring were collected by the supervising midwife and sent to the study coordination center at the Dr. von Haunersches Children's Hospital along with the declaration of consent and copies of relevant clinical documentation including the “maternity pass” and birth records. At six weeks postpartum, a standardized telephone interview with the mother was conducted by the study coordinator on participants’ and participants’ families medical history, trimester-specific nutrition during pregnancy, smoking during pregnancy, complications during pregnancy and at birth, and breastfeeding behavior. Thereafter, participating families received annual questionnaires on the psychomotor and physical development, including e.g. anthropometrical measurements conducted by the children’s pediatrician as part of the “well-child” visits, dietary habits and physical activity as well as e.g. information on parental education, employment and anthropometric measures. At age three to four months, families were invited for an infancy follow-up for anthropometrical check-up, collection of biomaterials (buccal smear, venous blood) and a questionnaire on pre-/peri-/postnatal factors as potential confounder. At five and eight to nine years of age, offspring were invited for an extensive cardiometabolic follow-up each, including analysis of body composition, blood pressure, heart rate variability, physical activity and collection of biomaterials. Mothers were invited two to three years post-partum for a maternal cardiometabolic follow-up with a focus on the development of prediabetes or T2D and examination of anthropometry, blood pressure, and vascular parameters such as intima media thickness and pulse wave velocity.

2.1.2 AdipoRiSc case-control study

The monocentric AdipoRiSc (Adiposity Risk Screening) case-control study comprises 60 normal weight (≥18.5 to <25.0 kg/m^2) and 61 obese (≥30.0 kg/m^2) women recruited between 2008 and 2009 to investigate biomarkers that predict obesity risk in clinically accessible surrogate cells, define a “molecular” signature that reflects early nutritive-metabolic dysregulations, and has been used to determine functional differences between peripheral

immune cells from obese and normal weight women (156-158). The study was approved by the Ethics Committee of the Technical University of Munich, Munich, Germany (project number: 2189/08). Written informed consent was obtained from all participants. Women were recruited in the Munich-Freising area via local newspapers, printed notes as well as the internet and local obesity support groups. Female sex, age between ≥ 20 and ≤ 45 years and a BMI between ≥18.5 to ≤24.9 kg/m^2 for controls and ≥30.0 to ≤46.5 kg/m^2 for cases with stable weight ± 3.0 kg for at least three months was required for inclusion. Metabolic, chronic, acute, neurological or mental diseases, usage of drugs with metabolic effects, smoking, pregnancy or lactation were exclusion criteria. After inclusion, women underwent clinical phenotyping including anthropometrical and cardiovascular measurements and blood sampling at the study unit of the Else Kroener-Fresenius Centre for Nutritional Medicine at the Technical University of Munich, Freising, Germany during a single morning visit after an overnight fast.

2.2 Data collection and definitions

2.2.1 Prenatal data of the PEACHES cohort

Maternal preconception BMI and GWG. Maternal self-reported weight and height as well as weight measurements during pregnancy were taken from the maternity pass issued at the first antenatal visit at the womens' gynecologist. Maternal preconception BMI was calculated by dividing the first measured weight in kg, if prior to gestational week 13, by the squared height in m. Otherwise self-reported preconception weight was used. BMI was categorized into normal weight (18.5-24.9 kg/m^2), obese (29.5-34.9 kg/m^2) and severely obese (≥35.0 kg/m^2). Total GWG was calculated by subtracting the last from the first recorded weight. Further, GWG was categorized into inadequate, adequate, excessive based on womens' preconception BMI according to the IOM (34) (Table 2).

GDM diagnosis: Information on womens' GDM status was obtained from their attending gynecologist or diabetologist. In the German health care setting, pregnant women usually undergo GDM testing between 24+0 und 27+6 weeks of gestation. We used results of this testing to classify women into GDM-negative or GDM-positive. As presented in Figure 8, either a one-step approach i.e. 75-g oral glucose tolerance test (OGTT)) or a two-step approach i.e. 50-g glucose challenge test (GCT) followed by a 75-g OGTT in case of a positive GCT result was performed. In the one-step approach, GDM testing was considered negative when none, and positive when one or more of the three measured glucose concentrations of the 75-g OGTT met or exceeded the reference values according to the International Association of the Diabetes Pregnancy Study Groups (IADPSG) criteria: fasting glucose ≥ 92 mg/dL, 1-h glucose ≥ 180 mg/dL, 2-h glucose ≥ 155 mg/dL (159). In the two-step approach, GDM testing was considered negative when the 1-h glucose of a 50-g GCT was < 140 mg/dL or, in case of a 1-

g glucose ≥ 140 mg/dL (160), none of the three glucose concentrations of a following 75-g OGTT met or exceed the reference values given by the IADPSG (159). In the two step approach, GDM testing was considered positive when 1-h glucose of a 50-g GCT was ≥ 140 mg/dL (160), and one or more of the three glucose concentrations of the following 75-g OGTT met or exceed the reference values given by the IADPSG (159).

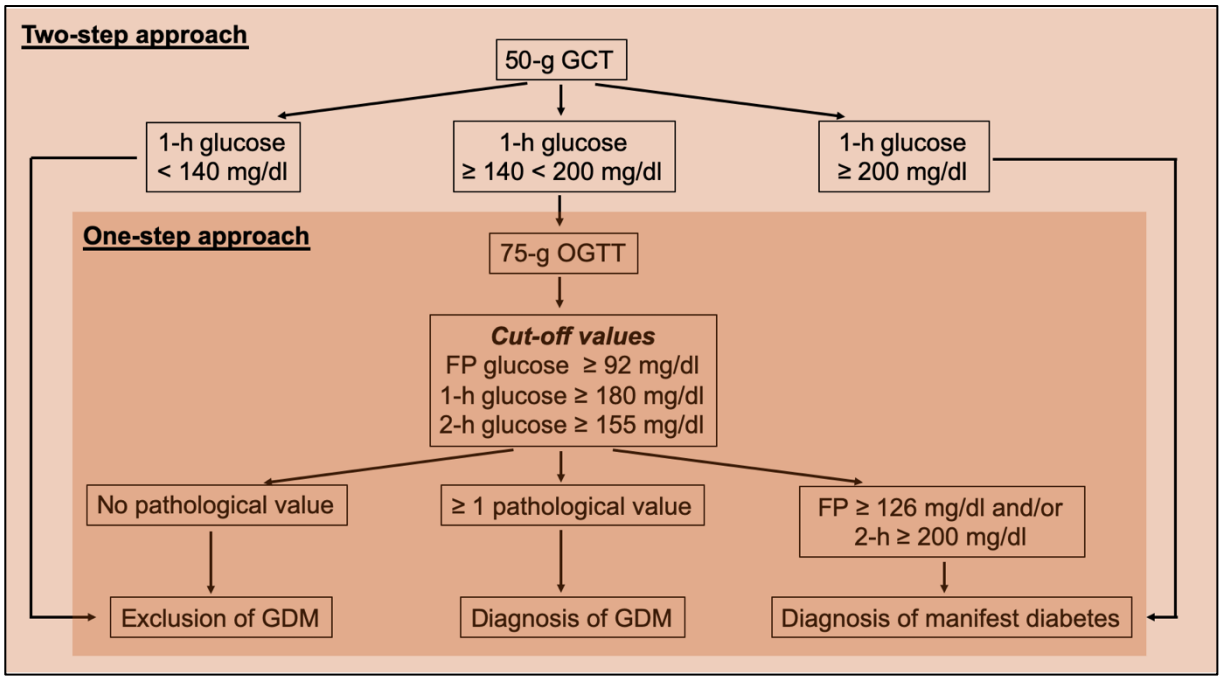

Figure 8: One-step and two-step approach for GDM diagnosis. To convert mg/dL to mmol/L, multiply by 0.0555. FP, fasting plasma; GCT, glucose challenge test; GDM, gestational diabetes; OGTT, oral glucose tolerance test. *Figure based on:* (159, 160).

Smoking during pregnancy: Information on smoking during pregnancy was obtained twice. During the standardized telephone interview six weeks postpartum, women were asked if and how many cigarettes they consumed during pregnancy and whether or not they stopped during pregnancy. Additionally, in the first questionnaire, sent to the women after the phone interview, the women were asked for the trimester-wise consumption of cigarettes. Reported smoking at any one of the two assessments was considered as “yes”, a negative response at both time points was considered as “no” for the variable “smoking any time during pregnancy” as described elsewhere (155, 161).

Arterial hypertension: A diagnosis of hypertension was performed retrospectively using blood pressure measurements from the maternity pass based on guidelines of the European Society Cardiology and Associations of Scientific Medical Societies in Germany (162-164). A woman was diagnosed as **normotensive** if all systolic and diastolic blood pressure measurements were <140 mmHg or <90 mmHg, respectively, throughout pregnancy, or, if only one measurement before and one measurement after 19 weeks and six days of gestation was

above or equal to one of these cut-off values. **Gestational hypertension** was diagnosed if blood pressure of two independent measurements after 19 weeks and six days of gestation was ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic for each measurement. Further, **gestational hypertension** was diagnosed if at one measurement before and at two or more independent measurements after 19 weeks and six days of gestation blood pressure was ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic for each measurement. A woman was diagnosed with **chronic hypertension** if blood pressure of two independent measurements before 19 weeks and six days of gestation was ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic in each measurement.

Folic acid: Information of maternal intake of folic acid was collected as part of the telephone interview.

2.2.2 Perinatal data from the PEACHES cohort

Gestational age: Information on gestational age (GA) was extracted from a copy of the birth record.

Maternal age at delivery: Information on maternal age at delivery was extracted from a copy of the birth record.

Mode of delivery: Information on mode of birth was extracted from a copy of the birth record. Mode of delivery was categorized into vaginal delivery, vacuum-assisted delivery, elective C-section or emergency C-section.

Offspring sex: Information on offspring sex was extracted from a copy of the birth record.

Perinatal infection**:** A short check list on signs of maternal infection including chorioamnionitis, maternal C-reactive protein (CRP) > 15 mg/L and intrapartal use of antibiotics was completed by the midwife. Chorioamnionitis and maternal CRP > 15 mg/L were considered as signs of maternal infection. UCB CRP was analyzed at an external laboratory (see 2.3.1). CRP > 5 mg/L was used as an indicator of neonate infection (156).

Late-pregnancy dysglycemia: Maternal HbA1c (%) at delivery was measured at the central laboratory (see 2.3.2). Late-pregnancy dysglycemia or high maternal HbA1c (%) was defined as maternal HbA1c (%) at delivery greater or equal to 5.7 % (39 mmol/mol) (154, 155).

2.2.3 Postnatal data of the PEACHES cohort

Breastfeeding: Information on breastfeeding were obtained from two questionnaires at six weeks and twelve months after delivery. Women were asked whether infants were

predominantly breast-fed (breastmilk as main source and complementary beverages such as water and tea), partially breast-fed (breastmilk and formula or baby food) or formula-fed (exclusively formula). Information was obtained week-wise for the first six weeks of life and month-wise for the first six months of life. We categorized data on breastfeeding (predominantly ≥1 month) into "yes" in case of predominantly breastfeeding ≥1 month or "no" in case of no or partial breastfeeding or predominantly breastfeeding <1 month as described elsewhere (155, 161).

BMI z-scores: Offspring's weight, height and head circumference were obtained from the regular well-child visits via questionnaire. The timeline of well-child visits in Germany are given in Table 4.

Table 4: Timeline of the well-child visits in the German health care setting

Well-child visit	Time frame
U1	At delivery
U2	3rd - 10th days of life
U3	4th - 5th weeks of life
U4	3rd - 4th months of life
U5	6th - 7th months of life
U6	10th - 12th months of life
U7	21st - 24th months of life
U7a	34th - 36th months of life
U8	46th - 48th months of life
U9	60th - 64th months of life

Offspring's weight and height measurements were used to calculate BMI z-scores based on WHO Child Growth Standards (165).

Socio-economic status: Information on parental school education and their occupational status before pregnancy was obtained via questionnaire to assess the socio-economic status (SES) based on an additive index (166). SES was categorized into "low", "medium" and "high" as described elsewhere (161).

Native language: Information on maternal native language was obtained via questionnaire.

2.2.4 Clinical data of the AdipoRiSc case-control study

BMI: During clinical phenotyping, performed by the same investigator to eliminate interindividual variations, womens' weight (kg) was measured using an electronic scale (BC 418 segmental body composition analyzer; Tanita, Sindelfingen, Germany) in underwear, without shoes. Height (m) was measured using a stadiometer and rounded to the nearest 0.1

cm (157, 158). BMI was calculated by dividing the weight by the squared height and categorized according to WHO (Table 1) (167).

Metabolically healthy: During the same phenotyping, womens' waist circumference (cm) was measured at the midpoint between the lower margin of the last rib and the top of the iliac crest according to WHO (168). Blood pressure (mmHg) measurement was performed in accordance with the 'Leitlinien der Deutschen Hochdruckliga e.V. DHL® - Deutsche Hypertonie Gesellschaft' (2008) (157, 158). Venous fasted blood samples were used to determine fasting glucose, TG, and HDL cholesterol (2.3.3). As women included in this study had no metabolic diseases, chronic and acute illnesses, women were defined as metabolically healthy if none of the components of the metabolic syndrome were detectable e.g. raised TG (≥150 mg/dL), reduced HDL cholesterol (< 50 mg/dL), raised blood pressure (systolic: ≥130 mmHg, diastolic: ≥85 mmHg), raised fasting blood glucose (≥100 mg/dL), and elevated waist circumference (≥ 80 cm) according to the Joint Interim Statement on the metabolic syndrome (169).

2.3 Biomaterials

2.3.1 UCB of the PEACHES cohort

UCB, combined (arterial and venous), was collected in tubes (BD Vacutainer®, Sarstedt, Heidelberg, Germany) immediately after delivery by the participants midwife. UCB was centrifuged at the one study center's laboratory for 10 minutes at 1,800 x g at room temperature (RT). Serum was stored at - 80 °C until analysis. A UCB serum aliquot was sent directly to the central laboratory (Laboratory Becker & Associates, Munich, Germany) for colorimetric analysis of high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and TG (AU 5800 Series Chemistry Analyzers, Beckmann, Munich, Germany). C-reactive protein (CRP) was analyzed by immunoturbidimetry (AU 5800 Series Chemistry Analyzers, Beckmann, Munich, Germany) and connecting-peptide (C-peptide) via luminescence immunoassay (Architect i2000, Abbott, Wiesbaden, Germany). The interassay coefficient of variation (CV), calculated by dividing the standard deviation (SD) by the mean, the limit of detection (LOD), which is the lowest analyte concentration that can be detected, but not necessarily quantified, and the limit of quantification (LOQ), which is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under predefined conditions (170) are provided for the analytes measured at delivery in Table 5.

Table 5: Interassay CV, LOD and LOQ of biochemical parameters from the central laboratory

	Interassay CV	LOD	LOQ
CRP	< 3.0 %	0.10 mg/L	0.20 mg/L

C-peptide	< 5.0 %	0.01 µg/L	0.10 µg/L
HDL cholesterol	< 2.5 %	0.10 mg/dL	2.0 mg/dL
LDL cholesterol	< 3.0 %	0.50 mg/dL	10 mg/dL
TG	< 2.0 %	1.00 mg/dL	10 mg/dL
HbA1c	< 3.5 %	-	1%
Glucose	< 2.5 %	2 mg/dL	10 mg/dL

CV, coefficient of variation; CRP, C-reactive protein; C-peptide, connecting peptide; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOD, limit of detection; LOQ, limit of quantification; TG, triglyceride.

2.3.2 Maternal blood of the PEACHES cohort at delivery

Maternal venous blood was collected in an ethylenediaminetetraacetic acid (EDTA)-containing tube (Sarstedt, Heidelberg, Germany) by the participants midwife shortly after delivery. The sample was sent to the Laboratory Becker & Associates, Munich, Germany for analysis of HbA1c using high performance liquid chromatography (HPLC) via cation-exchange chromatography (Tosoh G8 HPLC Analyzer, Tosoh Bioscience, Stuttgart, Germany). Interassay CV and LOQ are provided in Table 5.

2.3.3 Adult blood of the AdipoRiSc case-control study

Venous blood samples were collected after an overnight fast (at least 12 hours). Samples were centrifuged at 1200 x g for 10 min at RT and stored at -80 °C. An aliquot was sent to the same central laboratory (Laboratory Becker & Associates, Munich, Germany) as for the analyses of the PEACHES samples of routine biochemical parameters including TG, and HDL cholesterol as described before (2.3.1) and fasting glucose via an enzymatic UV-test (hexokinase) (AU 680 Series Chemistry Analyzers, Beckmann, Munich, Germany).

2.4 Metabolite extraction and quantification

All mass spectrometric analyses were performed in cooperation with the research group of Prof. H. Daniel at the Chair of Physiology, TUM, Freising, Germany, on a triple quadrupole QTRAP 5500 liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) system (Sciex, Framingham, MA, USA) equipped with a 1200 series binary pump (Agilent, Santa Clara, CA) and coupled to an HTC pal autosampler (CTC Analytics, Zwingen, Switzerland) using validated protocols.

2.4.1 Sample selection

400 UCB samples of offspring of normal weight, GDM-negative mothers (n = 112), obese GDM-negative mothers (n = 144) and obese GDM-positive mothers (n = 144) collected between 2011 and 2015 were used for LC-MS/MS of amino acids, acyl-carnitines, bile acids, and phospholipids. Selection was based on maternal preconception BMI (<24.9 kg/m^2 or ≥29.5 kg/m^2), GDM diagnosis, availability of pre-, peri- and postnatal data with preference for older children, offspring sex and pre-analytic criteria i.e. non-hemolytic, time until centrifugation

between 14 and 34 hours (median of time analyzed samples: 23 hours), availability of an adequate aliquot (50 µL needed).

For LC-MS/MS of amino acids and acyl-carnitines in adult blood, we chose samples of 20 metabolically healthy, normal weight women collected in 2009 (156-158).

2.4.2 Analytical set-up

UCB analyses were performed in six batches in 2016, and the metabolites choline, betaine, homocysteine (Hcys), sarcosine, and dimethylglycine were additionally analyzed in 2018 subsequent to findings of the biostatistical analyses. Samples were assigned to a "batch" according to the time until centrifugation as depicted in Table 6. Each batch, i.e. 96-well plate, contained an equal amount of samples of each group, i.e. samples of offspring of normal weight GDM-negative, obese GDM-negative and offspring of obese, GDM-positive mothers. For this purposes, 32 samples of offspring of normal weight, GDM-negative mothers were applied twice. Resulting concentrations for these samples were averaged. Samples of males and females were equally distributed on each plate. Adult blood was analyzed in one batch in 2017.

Table 6: Overview batches

Batch	Time until centrifugation in h (mean ± SD)	Number of samples per batch	Number of samples per group[a]	Sample of female offspring (%)
Cord blood				
Batch 1	14-19 (17.1 ± 1.5)	60	20	60
Batch 2	18-20 (19.5 ± 0.6)	60	20	42
Batch 3	21-22 (21.6 ± 0.5)	81	27	53
Batch 4	23-24 (23.5 ± 0.5)	81	27	53
Batch 5	25-28 (26.1 ± 1.0)	72	24	54
Batch 6	29-34 (31.5 ± 1.6)	78	26	47
Adult blood				
Batch 7	NA	20	NA	100%

[a]group: offspring of normal weight GDM-negative mothers, offspring of obese GDM-negative mothers, offspring of obese GDM-positive mothers

Five control plasma samples were analyzed with each batch to determine the CV. Distribution of samples on the 96-well plates are provided in Figure S 1.

2.4.3 Amino acids and acyl-carnitines

We used LC-MS/MS based on previously described methods for quantitative analysis of amino acid and derivatives (n = 37) and acyl-carnitine (n = 49) concentrations in PEACHES UCB (171, 172). A list of all amino acids, their derivatives and acyl-carnitines and their abbreviations is provided in Table S 1. In adult blood of the AdipoRiSC study, all but the metabolites analyzed in UCB in 2018 (choline, betaine, Hcys, sarcosine, and dimethylglycine) and all acyl-carnitines

but crotonylcarnitine (AC C4:1), 3-hydroxyisovalerylcarnitine and/or 3-hydroxy-2-methylbutyrylcarnitine (AC C5-OH), heptanoylcarnitine (AC C7:0), hydroxyoctanoylcarnitine (AC C8-OH) were analyzed. Thereby, n = 32 amino acids and n = 45 acyl-carnitines were available for comparison between UCB and adult blood. Briefly, 10 µL serum was dissolved in 500 µL ice-cold methanol containing 28 isotope-labeled internal standards (13 acyl-carnitines and 15 amino acids; Table 7), obtained from ChromSystems (Munich, Germany), except for Asparagine-15N2 and Glutamine-D5, which were obtained from Cambridge Isotope Laboratories Samples (Tewksbury, MA, USA),

Table 7: Internal standards used for LC-MS/MS of amino acids and acyl-carnitines

Amino acids	Acyl-carnitines
Alanine-D4	Carnitine-D9
Aspartic acid-D3	C2-Carnitine-D3
Glutamic acid-D5	C3-Carnitine-D3
Leucine-D3	C4-Carnitine-D3
Methionine-D3	C5-Carnitine-D9
Phenylalanine-D5	C5DC-Carnitine-D6
Tyrosine-D4	C6-Carnitine-D3
Valine-D8	C8-Carnitine-D3
Arginine-D7	C10-Carnitine-D3
Citrulline-D2	C12-Carnitine-D3
Glycine-13C2-15N	C14-Carnitine-D3
Ornithine-D6	C16-Carnitine-D3
Proline-D7	C18-Carnitine-D3
Asparagine-15N2	
Glutamine-D5	

LC-MS/MS, liquid chromatography coupled to-tandem mass spectrometry.

The samples were centrifuged at 10 min, 4 °C, 10,000 x g, their supernatants were collected thereafter and dried in nitrogen gas. As described by Gucciardi et al (173), we derivatized amino acids and acyl-carnitines to their butyl esters. In short, we added a mixture of 95% n-butanol and 5% acetylchloride (v/v) to the samples. Samples were subsequently incubated (60 °C for 15 min) while shaken (600 rpm; Eppendorf Thermomixer Comfort; Eppendorf, Hamburg, Germany). After being dried in nitrogen, we reconstituted the samples in a 300 µL mixture of methanol/water/formic acid (70/30/0.1% v/v). The chromatographic separation was achieved using a Zorbax Eclipse XDB-C18 column (length 150 mm, internal diameter 3.0 mm, particle size 3.5 µm; Agilent). The measurement of amino acids and acyl-carnitines was performed in two separate runs, each in positive ionization mode using scheduled multiple reaction monitoring (MRM). We generated a 10-point calibration of amino acid concentrations between 1 µM and 500 µM for absolute quantification of amino acids. We calculated acyl-carnitine concentrations based on analyte-to-internal standard area ratios and respective concentrations of internal standards. Data analysis was perfomed using Analyst 1.5.1® software (Sciex).

2.4.4 Bile acids

We used Quantitative LC-MS/MS analysis of bile acids (n = 16) was performed based on the method described by Frommherz (174). A list of all bile acids and their abbreviations is provided in Table S 1. In short, 10 µL of serum was dissolved in 500 µL methanol containing 15 isotope-labeled bile acid standards (Table 8), which were obtained from EQ Laboratories (Augsburg, Germany) and from Cambridge Isotope Laboratories.

Table 8: Internal standards used for LC-MS/MS of bile acids

Bile acids
Chenodeoxycholic acid-D4
Cholic acid-D4
Deoxycholic acid-D4
Glycochenodeoxycholic acid-D4
Glycocholic acid-D4
Glycodeoxycholic acid-D4
Glycolithocholic acid-D4
Glycoursodeoxycholic acid-D4
Lithocholic acid-D4
Taurochenodeoxycholic acid-D4
Taurocholic acid-D4
Taurodeoxycholic acid-D4
Taurolithocholic acid-D4
Tauroursodeoxycholic acid-D4
Ursodeoxycholic acid-D4

LC-MS/MS, liquid chromatography coupled to-tandem mass spectrometry.

The samples were centrifuged at 10 min, 4 °C, 12,000 x g and 400 µL of the supernatants were collected and evaporated to dryness in nitrogen gas. Dried samples were reconstituted in 100 µL methanol/water (50%/50% v/v) and shaken (5 min at 40 °C). A VDSpher PUR N1530E181VPH column (length 150 mm, internal diameter 3.0 mm, particle size 3.5 µm; Optilab GmbH, München, Germany) was used for chromatographic separation. We performed the measurement in negative ionization mode using scheduled MRM. A 10-point scale calibration curve of bile acid concentrations ranging between 1 nM and 2000 nM was generated for quantification. Data analysis was performed using Analyst 1.5.1® software (Sciex).

2.4.5 Phospholipids

Acyl-lysphosphatidylcholines (LysoPC a) (n = 18), diacyl-phosphatidylcholine (PC aa) n = 38), acyl-alkyl-phosphatidylcholine (PC ae) (n = 38) and un- (SM) (n = 10) and hydroxylated-sphingomyelin (SM(OH)) (n = 5), a total of n = 109 phospholipid species, were measured using direct flow injected MS/MS (175). A list of all phospholipids and their abbreviations is provided in Table S 1. The number of carbon atoms and double bonds for each species was given in the format “Cx:y” where x is the total number of carbon atoms and y the number of double

bonds. In LysoPC and PC, x is the sum of carbon atoms in both fatty acid chains. In SM, the number of carbon atoms and double bonds or the presence of hydroxyl group (OH) are noted only for the fatty acid in the amide bond under the assumption that the backbone is formed by sphingosine (d18:1). Briefly, 10 µL of serum was diluted with 190 µL 0.049% sodium chloride. 10 µL thereof were subsequently dissolved in 500 µL 5 mM ammonium acetic acid/methanol containing a mixture of phospholipids as internal standards (Table 9), which were obtained from Avanti Polar Lipids (Alabaster, AL, USA).

Table 9: Internal standards used for LC-MS/MS of phospholipids

Phospholipids
Lysophosphatidylcholine C9:0
Lysophosphatidylcholine C19:0
Phosphatidylcholine aa C14:0/C14:0
Phosphatidylcholine aa C20:0/C20:0
Sphingomyelin C6:0

LC-MS/MS, liquid chromatography coupled to-tandem mass spectrometry.

Samples were centrifuged (10 min, 10 °C, 3500 x g) and 400 µL of the supernatants were collected. We performed the measurement in positive ionization mode and calculated analyte concentrations based on analyte-to-internal standard area ratios and respective concentrations of internal standards. Data analysis was performed using MultiQuant® software (Sciex).

2.4.5.1 Isotope correction

We corrected the peak areas of phospholid species measured via direct-infusion MS/MS for their natural M+1 and M+2 isotopologues. Therefore, we calculated the isotopic distributions of individual lipid species using the Mass calculation tool provided by Lipid Maps (176). Specifically, for an analyte with mass *n*, we subtracted the calculated peak areas of the isotopologues *n* (M+1) and *n* (M+2) from the peak areas of the analytes with masses *n*+1 and *n*+2, respectively. Further, we added the calculated peak areas *n* (M+1) and *n* (M+2) to the peak area of the analyte with mass *n*.

2.5 Simultaneous RNA and DNA isolation from UCB

As only one PAXgene Blood RNA tube (PreAnalytiX, Hombrechtikon, Switzerland) was available per participant, simultaneous isolation of RNA and genomic DNA (gDNA) from PAXgene Blood tubes was established, as RNA and gDNA were needed for subsequent analysis. Isolation, and the following gene expression and methylation analysis were performed in cooperation with Dr. Soner Öner-Sieben (Department of General Pediatrics, Neonatology and Pediatric Cardiology, Division of Experimental Pediatrics and Metabolism, University Children's Hospital, Faculty of Medicine, Heinrich Heine University Düsseldorf, Düsseldorf, Germany).

2.5.1 Simultaneous isolation of RNA and DNA

RNA and gDNA were simultaneously isolated from PAXgene Blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland) containing UCB collected at birth and frozen at -80°C until analysis using the PAXgene Blood miRNA Kit (#763134, Qiagen, Hilden, Germany) for RNA and QIAmp DNA Blood Mini Kit (#51104, Qiagen, Hilden, Germany) for gDNA using a modified protocol from Kruhoffer et al. (177). In brief, tubes were allowed to thaw overnight at room temperature (RT) and gently swirled for homogenization. 1 mL was transferred to a 2 mL tube for isolation of gDNA. The remaining content was used for RNA isolation and centrifuged at RT for 10 min at 4600 rpm. Supernatant was removed and 4 mL RNAse-free water were applied to the pellet for dissolution. The tube was centrifuged as above. Supernatant was removed and 350 µL resuspension buffer was applied to the pellet. The following RNA isolation was carried out using an automated sample purification system (QIAcube, Qiagen, Hilde, Germany; protocol: PAXgene Blood miRNA Part A). The tube set aside for gDNA was centrifuged for 10 min at 14.000 x g at RT, the supernatant was discarded and the pellet was dissolved in 200 µL DPBS (Gibco, Thermo, Schwerte, Germany) with 4 µL RNase A (QIAcube, Qiagen, Hilde, Germany; QIAmp DNA Blood Mini, Blood or body fluid). Further processing was carried out on the automated purification system QIacube (Qiagen, Hilden, Germany). Quality and concentration of the isolated nucleic acids were assessed spectrophotometrically (NanoDrop, Thermofisher, Dreieich, Germany).

2.5.2 Polymerase chain reaction (PCR) analyses

2.5.2.1 PCR of gDNA

To verify gDNA isolation the gene very long chain acyl-CoA dehydrogenase (*ACADVL*) was amplified using different quantities of gDNA, i.e. 1, 4 and 10 µL, 20 µL REDTaq Ready Mix (Sigma-Aldrich, Taufkirchen, Germany), 1.5 µL forward primer (GTCCCTTCCCTGAACTTGCTAACC; 10 µM stock solution) and 1.5 µL reverse primer (CCTGGCCCCACCCAGCTCTGATTATCC; 10 µM stock solution) diluted with ddH_2O to a reaction volume of 40 µL. Polymerase chain reaction (PCR) was performed on a StepOnePlus PCR system (Thermo Fisher Scientific, Dreieich, Germany) under the following conditions: 1.) initial activating denaturation at 95°C for 5 min, followed by 45 cycles of 2.) 30 s denaturation at 95 °C, 3.) 30 s of annealing at 60 ° C, and 4.) 30 s elongation at 72 °C. A final elongation step was performed at 72 °C for 4 min before the reaction chamber was cooled down to 4 °C. The reaction product was separated by gel electrophoresis using 1% agarose gel.

2.5.2.2 Reverse transcription-PCR (RT-PCR)

For verification of RNA isolation we used the gene isovaleryl-CoA dehydrogenase (*IVD*). 100 ng of total RNA were utilized for complementary DNA (cDNA) synthesis using the QuantiTect RT Kit (Qiagen, Hilden, Germany) according to the manufacture guidelines. In short, gDNA

elimination reaction (2 µL gDNA wipeout buffer, template RNA, ad 14 µL RNase-free water) was prepared, incubated at 42 °C for 2 min and placed on ice. The gDNA elimination reaction was added to the reverse-transcription master mix (1 µL reverse-transcription master mix, 4 µL reverse transcription buffer, 1 µL reverse transcription primer mix) on ice. The mixture (20 µL) was then incubated for 15 mins at 42°C, for 3 mins at 95°C, subsequently placed on ice and diluted with ddH_2O to an endvolume of 100 µL. Samples for RT-PCR comprised 5 µL cDNA (0.5 ng/µL), 25 µl REDTaq Ready Mix (Sigma-Aldrich), 1 µL forward primer (GGAGATCGATCGCAGCAATGAGT; 10 µM stock solution) and 1 µL reverse primer (GGAAGCTCGGGATATCTCCTCCA; 10 µM stock solution) diluted with ddH_2O to a reaction volume of 50 µL. RT-PCR was performed on a StepOnePlus PCR system (Thermo Fisher Scientific, Dreieich, Germany) using the following conditions: 1.) initial activating denaturation at 95°C for 5 min, followed by 45 cycles of 2.) 15 s denaturation at 95 °C, 3.) 15 s of annealing at 58 °C, and 4.) 30s elongation at 72 °C. A final elongation step was performed at 72 °C for 4 min before the reaction chamber was cooled down to 4 °C. The reaction product was separated by gel electrophoresis using 1% agarose gel.

2.5.3 Pyrosequencing

Pyrosequencing was performed by Dr. Birgit Knebel at the German Diabetes Center (DDZ), Düsseldorf, Germany. An assay (Hs_CG06500161_02_PM_V1) analyzing methylation patterns in the sequence TAGTTTYGTYGGGT of the promotor region of the ATP Binding Cassette Subfamily G Member 1 (*ABCG1*) gene was used to compare the gDNA isolated from UCB in PAXgene, adult blood in PAXgene and adult blood in EDTA.

2.6 Gene expression analysis in UCB

2.6.1 Sample selection

Genes of interest were chosen based on the results of metabolite analysis. Genes were analyzed in a subgroup of “extremes”, i.e. offspring of severely obese mothers with either the “highest” or “lowest” concentrations of target metabolites compared to offspring of normal weight mothers with “normal” metabolite concentrations. Therefore, a cumulative distribution curve of target metabolites for n = 112 offspring of normal weight women was drawn to locate the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile in the distribution. Analogously, genes were analyzed in a subgroup of offspring of severely obese mothers with high maternal HbA1c (%) at delivery (≥ 5.7 %) and “highest” concentrations of target metabolites in offspring compared to offspring of severely obese mothers with normal maternal HbA1c (%) at delivery (< 5.7 %) and “normal” offspring metabolite concentrations. The cumulative distribution curve of target metabolites in n = 97 offspring of normal weight mothers with normal HbA1c (%) at delivery was analyzed to locate the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile in the distribution.

2.6.2 Real-time quantitative reverse transcription-PCR (qRT-PCR)

After isolation of RNA (2.5.1), we used 300 ng of total RNA for cDNA synthesis (QuantiTect RT Kit, Qiagen, Hilden, Germany) (2.5.2.2). The resulting 20µl cDNA were subsequently diluted with 180 µL ddH_2O. qRT-qPCR was performed in duplicates. Samples comprised 2 µL cDNA (1.5 ng/µL) with 0.5 µL of each primer (10 mM stock solution) and 10 µL SYBR Green Master mix (QuantiTect SYBR Green PCR Kit, Qiagen, Hilden, Germany) in a 20 µL reaction volume. Primer sequences are provided in Table 10.

Table 10: Primer sequences

Target gene	Forward primer	Reverse primer
ACADM	GGAAGCAGATACCCCAGGAAT	AGCTCCGTCACCAATTAAAACAT
ACADS	CGGCAGTTACACACCATCTAC	GCAATGGGAAACAACTCCTTCTC
ACADVL	TCAGAGCATCGGTTTCAAAGG	AGGGCTCGGTTAGACAGAAAG
ACAD8	AAACAGATGTGGGCGGGTCT	AGGCACACATGTTGTGGATGC
ALDH7A1	AGCCAAGGTTCTGGAGGACAAC	GAAGGACAGCAGGTTCACTCGT
BCAT1	GGTGGTGGGGACTTTTAAGGCTAA	TCTGAGGACCACTCCACCGT
BCKDHA	AGTACCGGGAGGCAGGTGTG	CAGCCGTAGTGGACAGGCAT
BCKDK	CATGCACGGCTTTGGCTTCG	GAAGCTTTCCTCCCGGCCAT
CPT1A	CCCCTCCAGTTGGCTTATCG	GACATGCAGTTGGCCGTTTC
CPT1B	CCTGCTACATGGCAACTGCTA	AGAGGTGCCCAATGATGGGA
CPT2	AAGAAGCAGCAATGGGCCAG	TCGTGGACAGGACATTGTGG
DLD	TGCAGAGCTGGAGTCGTGTG	ACTGTTACATCAGCATCAATCGGC
DNMT1	CGTGGAAGCCGGCAAAG	GTTGTGCTGAAGAAGCCGTC
DNMT3A	CCGATGCTGGGGACAAGAAT	CCCGTCATCCACCAAGACAC
DNMT3B	ACCTCGTGTGGGGAAAGATCA	CCATCGCCAAACCACTGGA
HPRT1	CAGCCCTGGCGTCGTGATTA	GCAAGACGTTCAGTCCTGTCC
IVD	CAGAGGCAGCTTCGTCAGACC	ATGCCCAATACGCCCAGGTT
MAT2A	GCTCCTTCGTAAGGCCACTT	GTCACAAATCTTATCTGGGTGGC
MTR	GTCGTCACCTGTGGAGAGC	CTTCAGACCTTCGGGTTGCG
PEMT	GGGGTTCGCTGGAACTTTC	GAGCCACTATGTAGGTGAGGG
SHMT1	AAGTTCGGGGTTTGGGGTTG	TGGTTCGAAGCTGCCTAGC
SHMT2	CCCTTCTGCAACCTCACGAC	TGAGCTTATAGGGCATAGACTCG
SLC25A20	TTAGAGAGGGCATCACGGGG	AGCTGAGCACATCTTCTGGG
UBE2D2	CATTCCCGAGCTATTCTGTT	CACAGTGGTCTCCAGCACTA

Forward and reverse primer used for qRT-PCR. qRT-PCR, Real-time quantitative reverse transcription polymerase chain reaction.

Primer were designed using the online NCBI-tool “Primer Blast”. Amplicon had at a length of 90-160 base pairs, at least one primer binding to an exon-exon-junction to exclude gDNA as template, and were designed to cover all transcript variants. PCR reactions were performed on a StepOnePlus PCR system (Thermo Fisher Scientific, Dreieich, Germany) under the following conditions: 1.) initial activating denaturation at 95°C for 15 min, followed by 44 cycles of 2.) 10 s denaturation at 95 °C, 3.) 20 s of annealing at 58 ° C, and 4.) 30 s elongation at 72 °C. The elongation step was used to collect fluorescence data. To determine the specificity of the primer for its template, a melt curve run was performed before the reaction chamber was cooled down to 4 °C.

We used the softwares Genorm (178) and Normfinder (179) to test a set of reference genes for accurate relative quantification. In both methods, a smaller value indicates a better stability of the tested reference gene. GeNorm further uses < 0.15 as cut-off for good stability, below this value there is no need for an additional reference gene. As all values using the GeNorm method were below <0.15, we used hypoxanthine phosphoribosyltransferase 1 (HPRT1) and ubiquitin conjugating enzyme E2 D2 (UBE2D2) for normalization as the most stable genes based on the combination of both software methods (Table 11). Reaction efficiencies for every run were determined *in silico* with LinRegPCR (Version 2017.1, (180)).

Table 11: Stability of reference genes

	Normfinder (stability value)	GeNorm (average expression stability)
18SRNA	0.072	0.586
28SRNA	0.180	0.826
ACTB	0.103	**0.476**
B2M	0.159	0.768
EEF1A1	0.092	0.620
GAPDH	0.118	0.544
HPRT1	**0.062**	**0.385**
PQIB	0.078	**0.385**
TBP	0.082	**0.438**
TFRC	0.161	0.703
UBE2D2	**0.051**	0.514

Normfinder (179) and GeNorm (178) were used to test stability of reference genes. Lower numbers indicate better stability in each method. Bold font emphasizes lowest values. ACTB, actin beta; B2M, beta-2-microglobulin; EEF1A1, eukaryotic translation elongation factor 1 alpha 1; GADPH, glyceraldehyde-3-phosphate dehydrogenase; HPRT1, hypoxanthine phosphoribosyltransferase 1; PQIB, intermembrane transport proteine PqiB; TBP, TATA-box binding protein; TFRC, transferrin receptor; UBE2D2, ubiquitin conjugating enzyme E2 D2.

2.7 Global methylation analysis

Global DNA methylation was assessed via 5-methylcytosine (5-mC) content utilizing an enzyme-linked immunosorbent assay (ELISA) Kit (MethylFlash Global DNA Methylation (5-mC) ELISA Kit, #P-1030, EpiGentek, BioCat, Heidelberg, Germany). According to the manufacturer's guidelines, 100 ng gDNA were used for each sample. Therefore, after gDNA isolation (2.5.1), concentration was set to 20 ng/µL, and 5 µL of gDNA were applied to the wells along with 100 µL of binding solution. Samples were measured in duplicates. For the negative control (NC), 100 µL of binding solution were combined with 2 µL unmethylated DNA (50 µg/mL) containing 0% 5-mC. For positive controls, different percentages (0.1% - 5%) of 5-mC methylated DNA were prepared to generate a standard curve. Therefore, positive control containing 5% 5-mC (at 5 ng/µL DNA) methylated DNA (50 µg/mL) was diluted with the negative control. 100 µL of binding solution were combined with 2 µL of methylated DNA containing 0.1-5% of 5-mC to wells. The covered plate was gently tilted and incubated at 37°C for 60 minutes. Thereafter, wells were washed with 150 µL diluted wash buffer (26 mL and 234

mL of distilled water and adjusted to a pH of 7.2 – 7.5) three times each. 50 µL of 5-mC detection complex solution (per 1 mL diluted wash buffer, 1 µL of 5-mC antibody, 1 µL of signal indicator and 0.5 µL of enhancer solution) was added to each well and incubated for 50 min at RT. The detection complex solution was removed and each well washed with diluted wash buffer five times. 100 µL of developer solution were added to each well, the covered plate was gently shaken and incubated at RT for 3-4 min. Once the enzyme reaction was evident from positive controls, reaction was stopped by adding 100 µL of stop solution to each well. The absorbance was read on a microplate reader at 450 nm within 15 minutes. A standard curve was generated by plotting the optical density (OD) of the positive controls against the respective 5-mC percentages (Figure 9). The percentage of methylated gDNA (5-mC) in the total gDNA was then calculated using the slope of the standard curve, (OD/1%) determined by linear regression, OD of the negative control, OD of the sample, and the amount of sample gDNA in ng (100 ng) according to the following formula:

$$\%5\text{-}\,\mathrm{mC} = \frac{\text{Sample OD} - \text{NC OD}}{\text{Slope x S}}\ \text{x}\ 100\%$$

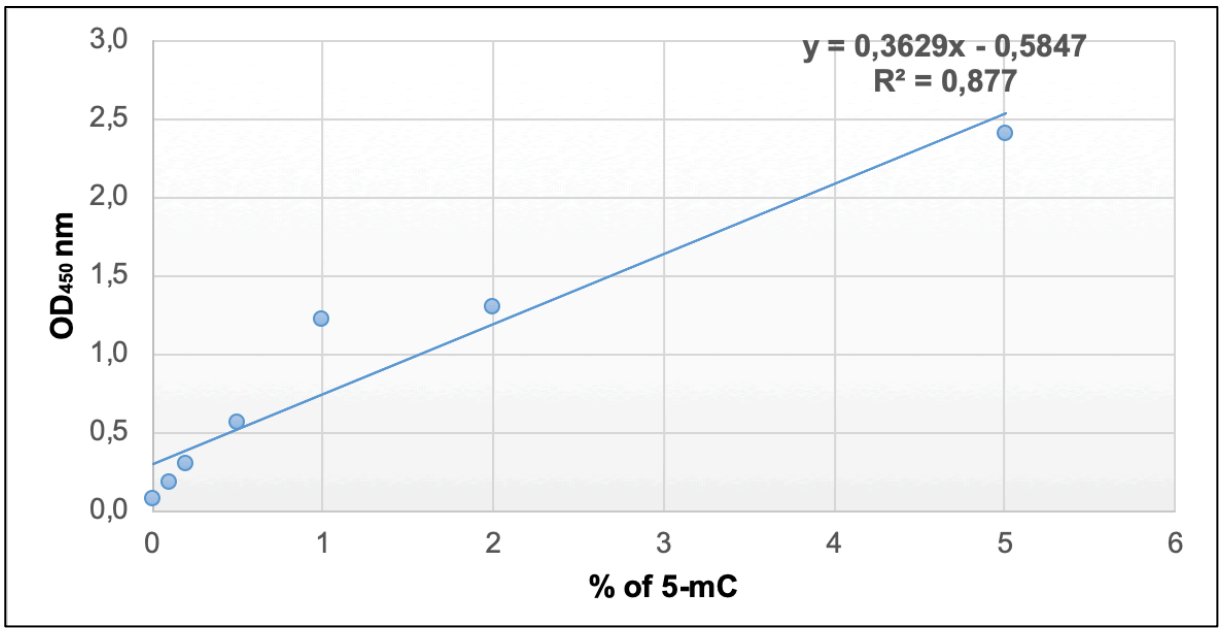

Figure 9: Standard curve. The x-axis represents the percentage (0.1 – 5.0%) of 5-mC of the positive controls, the y-axis represents the OD of the positive controls. 5-mC, 5-methylcytosine; OD, optical density.

2.8 Exposure and outcome variables

In accordance with the main aim of this thesis, a main explanatory variable and a main outcome variable were defined with regard to their impact on UCB metabolites. We investigated the main explanatory variable maternal preconception obesity (maternal preconception obesity and severe maternal preconception obesity vs. maternal preconception normal weight) on the outcome UCB metabolite concentrations. For confirmation, results were investigated using

mRNA expression and global DNA methylation analysis. Second, we investigated the main outcome variables preschool weight development and preschool weight outcome in relation to the explanatory variable UCB. Further, in subgroups, we analyzed the exposure of late-pregnancy dysglycemia within the group of obese mothers on the UCB metabolite profile. Besides, for the outcome UCB metabolites, we compared amino acids and acyl-carnitines concentrations between UCB of normal weight mothers and adult serum and analyzed the effect of several pre- and perinatal factors on UCB metabolite concentrations.

2.9 Statistical analysis

Statistical analysis was performed in cooperation with Dr. Gabi Kastenmüller (Institute of Computational Biology, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany), Dr. Christina Kunz (Department of Child Nutrition, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany), and Andreas Dunkel (Leibniz-Institute for Food Systems Biology, Technical University of Munich, Freising, Germany) in R unless otherwise noted. The following packages were used: “ggplot2”, “ropls”, “forestplot“, “calibrate“, “nlme”, “stats”, “multcomp“, ”biclust”, “VariancePartition”, “doParallel”.

2.9.1 Descriptive statistics

Descriptive statistical analysis was performed using Mann-Whitney-U for non-parametric, continuous variables, Student’s t-test for parametric, continuous and $\chi 2$ test for categorical variables. Bar plots were created in Graph Pad version 7.04.

2.9.2 Analyses of metabolite data for comparison of UCB with adult blood

2.9.2.1 Preprocessing of UCB metabolite data

Due to different time points of analysis of UCB and adult blood samples, preprocessing for this analysis differed slightly from preprocessing of the overall UCB dataset. Extreme outliers exceeding the range [mean - 4*SD, mean + 4*SD] were interpreted as measurement errors or contamination, and counted as missing. Values lower than the LOD and missings denoting extreme outliers were imputed simultaneously by the k (k=10) nearest neighbors approach suggested by Do et al. (181). No samples had to be excluded based on the metabolite measurements. Thus, samples of n = 112 offspring of normal weight, healthy (GDM-negative) mothers vs. samples of n = 20 metabolically healthy female adults were available for statistical analysis.

We performed median normalization, i.e., we divided single metabolite values and derived metabolite values by the median of corresponding (derived) control plasmas per plate. Log2-transformed values of normalized (derived) metabolites were used for all further analyses. Due

to an interassay CV ≥ 20%, we excluded the following metabolites from analysis: methacrylylcarnitine (AC 2-M-C3:1), undecanoylcarnitine (AC C11:0), tridecanoylcarnitine (AC C13:0), tetradecenoylcarnitine (AC C14:1), pentadecanoylcarnitine (AC C15:0), Heptadecanoylcarnitine (AC C17:0), methylglutarylcarnitine (AC C5-M-DC), Hexenoylcarnitine (AC C6:1), nonanoylcarnitine (AC C9:0), α-aminoadipic acid (AADP), anserine, asparagine (Asn), carnosine, c-cysteine (Cys-Cys), γ-aminobutyric acid (GABA), 15-Methylhexadecanoylcarnitine (AC iso-C17:0).The analysis set comprised single metabolites (n = 61) analyzed in UCB and adult blood with interassay CV ≤ 20%, biologically relevant sums and ratios of those metabolites (n = 28). Further, in univariate analyses, all pairwise ratios out of amino acids and all pairwise ratios out of acyl-carnitines (n = 1573) were analyzed. Reported were only the results of single and biological relevant sums and ratios.

2.9.2.2 Univariate analysis

We used univariate linear regression models to investigate differences in metabolite concentrations between UCB of offspring of normal weight mothers and female adult blood. were calculated to identify Differences in metabolites, sums and ratios between the two groups were based on β-estimates, 95% CI and q-values. For single metabolites and relevant sums and ratios, test results with q-values < 0.05 were considered statistically significant. As a sensitivity analysis, univariate linear regression models were run in female UCB vs. female adult blood.

2.9.2.3 Hierarchical clustering

We performed hierarchical (agglomerative) cluster analysis. Spearman's rank correlation was used as similarity measure and the average linkage as agglomeration method. Heatmaps including dendograms were provided to visualize the clustering result. Hierarchical clustering was performed using the "hclust" function. As a sensitivity analysis, univariate linear regression models were run in female UCB vs. female adult blood.

2.9.2.4 Multiple linear regression

In UCB samples of offspring of normal weight, healthy mothers, multiple linear regression models were applied to single and relevant derived metabolite values in order to investigate the influence of the categorical offspring sex (male/female), mode of delivery (vaginal/C-section), GWG (inadequate/adequate and excessive/adequate), maternal HbA1c (%) at delivery (≥ 5.7 % vs. < 5.7 %), and batch (plates 1-6) and the continuous variables GA and birth weight. Simultaneous p-values were derived for each model using the fuction "glht" and additionally adjusted by the method of Benjamini and Hochberg.

2.9.2.5 Biclustering

We performed biclustering to simultaneously cluster samples and metabolites to find homogeneous subgroups, i.e. “biclusters”, of metabolites and samples. “Biclusters” may overlap and thus might better reflect the biological “reality” as a metabolite might be part of more than one pathway. We perform Plaid model biclustering as described by Turner et al. (182). As a sensitivity analysis, univariate linear regression models were run in female UCB vs. female adult blood.

2.9.3 Analyses of UCB metabolite data, exposures and offspring outcome

2.9.3.1 Preprocessing of UCB metabolite data

Out of the total of 211 metabolites measured in UCB, the two phospholipids PC aa C30:2 and PC aa C32:2 had to be excluded from further analysis due to missing values >90% of the 400 UCB samples, resulting n = 398 samples for statistical analysis. We calculated the interassay CV by dividing the SD by the mean for the remaining metabolites measured in UCB based on the measurements obtained for 30 aliquotes of reference plasma i.e. 5 per batch. The following 19 metabolites showed an interassay CV of >25%: GABA, AADP, anserine, Asn, carnosine, (AC 2-M-C3:1), AC C4:1, AC C7:0, (AC C5-M-DC), (AC C9:0), AC C11:0, AC C13:0, AC C14:1, LysoPC a C26:0, PC aa C24:0, PC aa C36:0, PC ae C38:2, PC ae C44:3, SM C26:0. We did not exclude any of these metabolites, because the measured concentrations of the respective metabolites in UCB were by at least one order of magnitude higher compared to reference plasma. Very low concentrations of a metabolite in reference plasma might result in a high interassay CV. If concentrations for the metabolite were distinctly higher in uCB, uncertainty of measured suggested by a high interassay CV does not necessarily hold true for UCB. Thus, concentrations in UCB were still investigated so useful information was not wrongly discarded. Metabolite concentrations were log2 transformed. We tested samples for multivariate outliers using the Mahalanobis distance (critical $\chi 2$ value p-values < 0.05), which lead to the removal of two samples due to missingness in phospholipid measurements leading to n = 398 samples for analysis. In all statistical analyses, data points exceeding the range [Mean - 4*SD, Mean + 4*SD] were omitted for each metabolite. Metabolite characteristics including mean and SD of metabolite concentrations of original and log2-transformed data of the entire dataset and mean and SD of log-transformed metabolite concentrations in offspring of normal weight mothers (≥18.5–24.9 kg/m^2) for reference, interassay CV, missings, LOD and LOQ for all 209 metabolites, TG, HDL cholesterol and LDL cholesterol are provided in Table S 1. Explanations of sums and ratios (n = 71) and their equivalent mean and SDs for 71 are provided in Table S 2. For each metabolite, the mean concentration of the reference plasma was divided by the mean signal-to-noise ratio of the reference plasma and multiplied by 3 for LOD and by 9 for LOQ (172).

2.9.3.2 Partition plots

Violin plots were performed using the following pre-and perinatal factors on the dataset to investigate the variance of metabolite concentrations in UCB. The categorical variables were batch (plates 1-6), mode of delivery (vaginal/vacuum-assisted/elective C-section/emergency C-section), offspring sex (male/female), intrapartum antibiotics (yes/no), SES (low/middle/high), GDM diagnosis (negative/positive), maternal CRP >15 mg/l (yes/no), folic acid (yes/no), hypertension (chronic/gestational/normotensive), smoking during pregnancy (yes/no). The continuous variables were maternal HbA1c (%) at delivery, GA, birth weight, GWG, maternal preconception BMI, maternal age.

2.9.3.3 Hierarchical clustering

Hierarchical (agglomerative) cluster analysis was performed using Spearman's rank correlation as similarity measure and the average linkage as agglomeration method to depict similarities between pre- and perinatal influencing factors on the explainable variance in UCB metabolites in offspring. Heatmaps including dendograms visualize the clustering result. Hierarchical clustering was performed using the "hclust" function.

2.9.3.4 Multivariate analysis

Partial latent structure-discriminant analysis (PLS-DA) was used exploratively, to determine exposure variables for subsequent analysis before preprocessing of the data set. Orthogonal partial latent structure (OPLS) regression was used to organize the metabolite data according to maternal preconception BMI after data was preprocessed. Categories were introduced thereafter for visualization.

2.9.3.5 Multiple linear regression

Effect of maternal obesity on UCB metabolite profile: We conducted multiple linear regression models with maternal preconception BMI group (obese (n = 128), severely obese (n = 159) as explanatory variable and UCB metabolite as outcome variable. Offspring of normal weight mothers (n = 111) were used as reference group. The model was adjusted for the categorical variables batch (plates 1-6), mode of delivery (vaginal/vacuum-assisted/elective C-section/emergency C-section), offspring sex (male/female) and the continuous variables birth weight, GWG, GA and maternal HbA1c (%) at delivery. Offspring with missing data on any of the potential confounders were excluded from the analysis. Analyses were applied to all 209 metabolites, as well as 71 sums and ratios, HDL cholesterol and LDL cholesterol and TG . In addition, the model was run after the dataset was stratified by offspring sex. We adjusted the p-values by the method of Benjamini and Hochberg, which controls the false discovery rate (FDR) to correct for multiple testing (183). Results are reported as significant for a q-value (the adjusted p-value) < 0.05. Concentrations were expressed as fold changes to visualize differences in metabolite concentrations according to maternal BMI group in volcano plots. β-

estimates of male and female offspring were compared by substracting the β-estimates of males from the β-estimates of females divided by the square root of squared β-estimates of males added to the squared β-estimates of females.

Effect of late-pregnancy dysglycemia in obese pregnancies on UCB metabolite profile: The dataset was stratified by maternal BMI. Within offspring of obese mothers (n = 287) multiple linear regression models were conducted with late-pregnancy dysglycemia i.e high maternal HbA1c (%) at delivery (≥ 5.7 %; n = 106) as explanatory variable and UCB metabolite as outcome variable. Offspring of obese mothers with normal HbA1c (%) at delivery (< 5.7 %; n = 181) were used as reference group. We adjusted for the categorical variables batch (plates 1-6), mode of delivery (vaginal/vacuum-assisted/elective C-section/emergency C-section), offspring sex (male/female) and the continuous variables birth weight, GWG, and GA. Offspring with missing data on any of the potential confounders were excluded from the analysis. Analyses were applied to all 209 metabolites, as well as 71 sums and ratios, HDL cholesterol and LDL cholesterol and TG. Results are reported as significant for p-value < 0.05 as none of the associations remained significant after adjusting for multiple testing.

Effect of UCB metabolite profile on offspring BMI z-score development: BMI z-scores were calculated based on World Health Organization (WHO) Child Growth Standards (165) and the log2-transformed metabolite concentrations were scaled to a mean of 0 and SD of 1. The dataset was stratified by maternal preconception BMI group (normal weight (n = 111) and obese (≥29.5 kg/m^2, n = 287). Multiple linear regression models were conducted with UCB metabolite as explanatory variable and the offspring BMI z-score slope as outcome variable. The slope from one to four years of age was estimated via linear regression, as changes of offspring BMI z-scores from one to four years could be considered linear in the PEACHES cohort as previously shown (155). The slope was based on estimations per child per year, if, at least two BMI z-score measurements were available, which applied to all 398 samples. In the main model (Model 1), we adjusted for the categorical variables smoking anytime during pregnancy (yes/no), predominantly breastfeeding ≥1 month (yes/no), SES at birth (low/middle/high) and the continuous variable GWG. Offspring with missing data on any of the potential confounders were excluded from the analysis. Analyses were applied to all 209 metabolites, as well as 71 sums and ratios, HDL cholesterol and LDL cholesterol and TG. Results were reported as significant for a p-value < 0.05, as none of the associations remained significant after adjusting for multiple testing. Analyses were repeated after data was stratified by offspring sex. For metabolites with p-values < 0.05 in offspring of normal weight and/or obese mothers associations were displayed in forest plots using scaled β-estimates and standard error (SE). For sensitivity analyses, we estimated models which were additionally

corrected for maternal preconception BMI (normal weight and obese, ≥29.5 kg/m^2) (Model 1a) and maternal HbA1c (%) at delivery (Model 2) in metabolites with p-values < 0.05. The analysis was repeated using the residuals of metabolites that is the log2-transformed metabolite concentration corrected for linear effects of batch (plates 1-6), offspring sex (male/female), GA, birth weight, and mode of delivery (vaginal/vacuum-assisted/elective C-section/emergency section) (Model 3).

For comparison of the development of BMI z-scores according to UCB isovalerylcarnitine (AC 3-M-C4:0) concentrations, AC 3-M-C4:0 tertiles based on the concentrations of all offspring (n = 398), regardless of maternal preconception BMI and offspring sex, were calculated and the mean BMI z-scores within the first (lowest) and third (highest) tertile at one, two, three and four years was plotted in offspring of obese (n = 287) and offspring of normal weight mothers (n = 111).

2.9.3.6 Linear mixed effect models

Effect of UCB metabolite profile on offspring BMI z-scores at different preschool ages: The dataset was stratified by maternal preconception BMI group (normal weight (n = 111) and obese (≥29.5 kg/m2; n = 287). Linear mixed effect models were estimated with BMI z-scores at ages one, two, three, and four years as outcome, metabolite and age (at weight/height measurement) as fixed effects, and assuming a random intercept. All offspring were included in the model regardless of availability of measurements for all time points. We included the following covariates in the main model (Model 1) GWG, smoking anytime during pregnancy (yes/no), predominantly breastfeeding ≥1 month (yes/no), and SES at birth (low/middle/high). Offspring with missing data on any of the potential confounders were excluded from the analysis. Analyses were applied to all 209 metabolites, as well as 71 sums and ratios, HDL cholesterol and LDL cholesterol and TG. Results were reported as significant for a p-value < 0.05 as none of the associations remained significant after adjusting for multiple testing. Analyses were repeated after data was stratified by offspring sex. For metabolites with p-values < 0.05 in offspring of normal weight and/or obese mothers associations were displayed in forest plots using scaled β-estimates and SE. For sensitivity analyses, we estimated models which were additionally corrected for maternal preconception BMI (normal weight and obese, ≥29.5 kg/m2) (Model 1a) and maternal HbA1c (%) at delivery (Model 2) in metabolites with p-values < 0.05. The analysis was repeated using the residuals of metabolites that is the log2-transformed metabolite concentration corrected for linear effects of batch (plates 1-6), offspring sex (male/female), GA, birth weight, and mode of delivery (vaginal/vacuum-assisted/elective C-section/emergency section) (Model 3).

In an additional analysis of glycoursodeoxycholic acid (GUDCA), we further adjusted Model 1 for intrapartum antibiotic treatment, maternal preconception BMI and maternal HbA1c (%) at delivery. Model 1 was also run after the dataset was stratified by predominantly breastfeeding ≥1 month (yes/no). Further, GUDCA tertiles were calculated based on the concentrations of all offspring. BMI z-scores of offspring at age four years, who were breastfeed predominantly ≥1 months (n = 184), were depicted in boxplots to visualize the distribution of maternal preconception BMI group and intrapartum antibiotic treatment within these groups. Offspring with missing data on intrapartum antibiotic treatment were excluded from analysis. For comparison of BMI z-scores development according to GUDCA concentrations, the median BMI z-scores within the first (lowest) and third (highest) tertile at one, two, three and four years was plotted in offspring of obese (n = 287) and offspring of normal weight mothers (n = 111).

2.9.4 Gene expression analysis

Expression and statistical analysis was conducted with the REST2009 software (184) and excel version 16.16.22. P-values ≤ 0.05 were considered statistically significant.

2.9.5 Global DNA-Methylation

A two-tailed Student's t-test was performed to test statistical significance using Graph Pad version 7.04. P-values ≤ 0.05 were considered statistically significant.

3 Results

3.1 Comparison of metabolites in UCB and adult blood

To compare the quality and quantities of metabolite profiles of newborns with those of adults, the metabolite concentrations in UCB and adult blood were studied. UCB of female (n = 60) and male (n = 52) offspring of normal weight, healthy mothers was used to compare the concentrations of amino acids and acyl-carnitines as well as their sums and ratios to blood of metabolically healthy, female participants (n = 20) of the AdipoRiSc study.

3.1.1 Characteristics of the AdipoRiSc participants

We used venous blood of middle-aged, Caucasian, non-pregnant and non-smoking women with a mean BMI of 21.7 kg/m^2 for analysis. Women were metabolically healthy defined by the parameters of the metabolic syndrome: waist circumference, fasting glucose, TG, HDL cholesterol, and blood pressure. Clinical and anthropometrical characteristics of women of the AdipoRiSc study (156) are presented in Table 12. We studied n = 20 Caucasian, normal weight, metabolically healthy, non-pregnant, non-smoking participants. Neonates were n = 112 offspring from the PEACHES study whose mothers were normal weight (mean BMI: 22.0 ± 1.6 kg/m^2) and healthy (GDM-negative). They were delivered at term without clinical infection, mostly vaginally (Table 15). Offspring sex was equally distributed among the analyzed neonates. Apart from significantly higher birth weight among male (mean: 3621.2 ± 420.5 g) compared to female (mean 3288.9 ± 432.0 g) offspring, no differences were detected.

Table 12: Characteristics of the participants of the AdipoRiSc (n = 20)

	AdipoRiSc women
Age, years	37.1 (3.7)
BMI, kg/m^2	21.7 (1.6)
Waist circumference, cm	73.6 (3.1)
Fasting glucose, mg/dL	79.5 (10.6)
2-h post load glucose, mg/dL	113.0 (16.1)
TG, mg/dL	79.3 (31.3)
HDL cholesterol, mg/dL	70.3 (12.0)
Systolic blood pressure, mmHg	100.7 (9.4)
Diastolic blood pressure, mmHg	65.7 (5.2)

Data are mean (SD). AdipoRiSc, Adiposity Risk Screening; BMI, body mass index; HDL, high-density lipoprotein; SD, standard deviation; TG, triglycerides.

3.1.2 Differences in amino acid and acyl-carnitine concentrations in offspring UCB compared to adult blood

Concentrations of amino acids and acyl-carnitines in UCB were compared to those in female adult blood using univariate linear regression. In addition to amino acid and acyl-carnitine concentrations, and their biological sums and ratios, we analyzed ratios of all amino acids and ratios of all acyl-carnitines to account for the batch effect and provide better comparability of

UCB and adult samples. Overall, 1271 entities differed between UCB and adult blood. In the following, only single metabolite concentrations and biological sums and ratios are discussed. Out of 61 analyzed single metabolites, 50 differed significantly between UCB and female adult blood after adjustment for multiple testing (Table 13).

Table 13: Beta-estimates (95% CI) for metabolite concentrations, ratios and sums in UCB of offspring of both sexes compared to female adult blood

	UCB vs. female adult blood
Metabolites	β-estimate (95% CI)
Acyl-carnitines	
AC 2-M-C3:0	not significant
AC 2-M-C4:0	0.559 (0.311; 0.807)***
AC 3-M-C4:0	-0.566 (-1.011; -0.121)*
AC C0	-0.822 (-0.957; -0.688)***
AC C10:0	-2.747 (-2.998; -2.496)***
AC C10:1	-1.849 (-2.074; -1.624)***
AC C12:0	-1.708 (-1.994; -1.422)***
AC C12:1	-1.855 (-2.144; -1.565)***
AC C14:0	0.580 (0.279; 0.881)***
AC C16:0	1.132 (0.855; 1.408)***
AC C16:1	0.849 (0.526; 1.714)***
AC C18:0	not significant
AC C18:1	0.384 (0.115; 0.653)**
AC C18:2	1.326 (1.016; 1.636)***
AC C2:0	-0.392 (-0.602; -0.181)***
AC C3-DC	0.313 (0.144; 0.482)***
AC C3-M-DC	not significant
AC C3:0	0.296 (0.083; 0.510)**
AC C4:0	not significant
AC C4-DC	0.645 (0.434; 0.856)***
AC C4-OHa	0.991 (0.679; 1.303)***
AC C4-OHb	0.515 (0.028; 1.003)*
AC C5:1	-0.479 (-0.719; -0.239)***
AC C5-DC	not significant
AC C6:0	not significant
AC C6-DC	1.028 (0.644; 1.412)***
AC C6-OHa	0.559 (0.297; 0.820)***
AC C6-OHb	not significant
AC C7-DC	not significant
AC C8:0	-1.929 (-2.174; -1.684)***
AC C8:1	-1.151 (-1.515; -0.786)***
AC iso-C11:0	-1.810 (-2.148; -1.472)***
AC iso-C13:0	-0.714 (-1.069; -0.360)***
AC iso-C15:0	not significant
AC iso-C9:0	-1.564 (-2.130; -0.999)***
Amino acids	
1-M-His	0.438 (0.243; 0.634)***
3-M-His	not significant
AAB	0.265 (0.035; 0.495)*
Ala	1.077 (0.918; 1.236)***

Arg	-1.363 (-1.693; -1.033)***
Asp	2.608 (2.429; 2.787)***
BAIB	not significant
Cit	-0.769 (-0.928; -0.609)***
Gln	-0.245 (-0.382; -0.107)***
Glu	3.293 (3.154; 3.432)***
Gly	0.799 (0.661; 0.938)***
His	0.918 (0.788; 1.048)***
Ile	0.696 (0.551; 0.841)***
Leu	0.341 (0.193; 0.489)***
Lys	1.339 (1.213; 1.464)***
Met	1.035 (0.924; 1.146)***
OH-Pro	1.717 (1.547; 1.886)***
Orn	1.733 (1.591; 1.875)***
Phe	0.942 (0.843; 1.040)***
Pro	0.494 (0.373; 0.614)***
Ser	0.949 (0.815; 1.082)***
Thr	1.428 (1.253; 1.603)***
Trp	0.420 (0.309; 0.530)***
Tyr	0.553 (0.447; 0.660)***
Val	0.311 (0.199; 0.423)***
β-Ala	-0.541 (-0.944; -0.138)*
Ratios	
Acyl-carnitines	
AC C12:0/AC C10:0	1.035 (0.853; 1.217)***
(AC C16:0+AC C18:0)/AC C0	1.845 (1.557; 2.132)***
(AC C2:0+AC C3:0)/AC C0	0.452 (0.297; 0.606)***
AC C14:0/AC C16:1	-0.262 (-0.407; -0.116)***
AC C2:0/AC C0	0.418 (0.251; 0.585)***
AC C3:0/AC C4:0	not significant
Amino acids	
Asn/Gln	1.333 (1.177; 1.488)***
Asp/Asn	1.546 (1.384; 1.708)***
Cit/Arg	0.571 (0.210; 0.932)**
Cit/Orn	-2.518 (-2.671; -2.366)***
Fisher	-0.293 (-0.397; -0.188)***
Glu/Gln	3.555 (3.340; 3.769)***
Orn/Arg	3.050 (2.673; 3.427)***
Orn/Ser	0.785 (0.660; 0.909)***
Tyr/Phe	-0.367 (-0.459; -0.274)***
Sums	
Acyl-carnitines	
AC	-0.751 (-0.882; -0.620)***
AC C16:0+AC C18:0	1.024 (0.753; 1.295)***
AC C2:0+AC C3:0	-0.357 (-0.555; -0.158)***
Even. AC	-0.314 (-0.498; -0.130)**
Long. AC	0.845 (0.578; 1.112)***
Medium. AC	-1.599 (-1.843; -1.354)***
Odd. AC	not significant
Amino acids	
AA	0.861 (0.774; 0.948)***
Aromatic AA	0.669 (0.586; 0.752)***

BCAA	0.405 (0.286; 0.525)***
Essential AA	0.908 (0.818; 0.997)***
Glucogenic AA	0.976 (0.855; 1.098)***
Nonessential AA	0.767 (0.669; 0.866)***

Data are presented as β-estimate (95% CI) of acyl-carnitine and amino acid concentrations (µmol/L) in UCB (n = 112) compared to female adult blood (n = 20).Data are based on univariate linear regression models. Statistical significance is based on p-values adjusted for multiple testing by the method of Benjamini-Hochberg. * = q-value < 0.05, ** = q-value < 0.01 *** = q-value < 0.001. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. AA, amino acids; AC, acyl-carnitines; CI, confidence interval; UCB, umbilical cord blood.

Of the 26 amino acids analyzed in UCB and female adult blood, 24 differed significantly. All of the differential amino acids but arginine (Arg), β-alanine (β-Ala), citrulline (Cit) and glutamine (Gln) and the sums of amino acids showed higher concentrations in UCB compared to female adult blood. Product-to-substrate ratios related to the enzymatic activities of arginase (ornithine (Orn)/Arg) (185, 186), phosphoglycerate dehydrogenase (Orn/Ser) (187), nitric oxide synthase (Cit/Arg) (185, 188), glutaminase (glutamate(Glu)/Gln) (189), asparagine synthetase (aspartate (Asp)/Gln) (190), and asparaginase (Asp/Asn) (190) had higher, those related to the enzymatic activities of ornithine carbamoylphosphate transferase (Cit/Orn) (185, 191), phenylalanine hydroxylase (tyrosine (Tyr)/phenylalanine (Phe)) (185, 192) and the fisher ratio (185, 193) had lower concentrations in UCB compared to female adult blood. Of the 35 acyl-carnitines, 26 differed significantly between UCB and adult blood. 13 acyl-carnitines showed higher and 13 others showed lower concentrations in UCB compared to female adult blood. The group with higher concentrations in UCB contained mainly short- and long-chain acyl-carnitines including saturated, unsaturated, hydroxylated and dicarboxylic species. Lower concentrations were observed for medium-chain saturated and monounsaturated acyl-carnitines, as well as in acetylcarnitine (AC C2:0) and free carnitine (AC C0). All product-to-substrate ratios related to β-oxidation i.e. (AC C2:0 + propionylcarnitine (AC C3:0)) / AC C0, related to overall β-oxidation activity (185), dodecanoylcarnitine (AC C12:0) / decanoylcarnitine (AC C10:0), related to ACADM activity (187), AC C2:0 / AC C0, related to β-oxidation activity of even numbered fatty acids (185), and (hexadecanoylcarnitine (AC C16:0) + octadecanoylcarnitine (AC C18:0))/AC C0, related to CPT1 activity (185, 186) had higher concentrations in UCB, whereas the ratio tetradecanoylcarnitine (AC C14:0)/ hexadecenoylcarnitine (AC C16:1), related to activity of stearoyl-CoA desaturase (187) had lower concentrations in UCB. As a sensitivity analysis, we compared only female UCB to female adult blood (Table S 3). With the exception of the four metabolites α-aminobutyric acid (AAB), AC 3-M-C4:0, octadecenoylcarnitine (AC C18:1), 3-hydroxybutyrylcarnitine (AC C4-OHb), all metabolites that differed between UCB of both sexes and female adult blood also differed between UCB of female offspring and female adult compared. Magnitude and direction of the β-estimates were also similar.

Next, hierarchical clustering was performed on samples and metabolites to generate a heatmap with dendograms drawn on the x-axis (samples) and y-axis (metabolites) based on similarities of samples and metabolites (Figure 10). The clustering of samples (x-axis) is based on the similarity of samples with regard to their metabolite profile. The clustering of metabolites (y-axis) is based on the similarity of metabolite concentrations with regard to the samples.

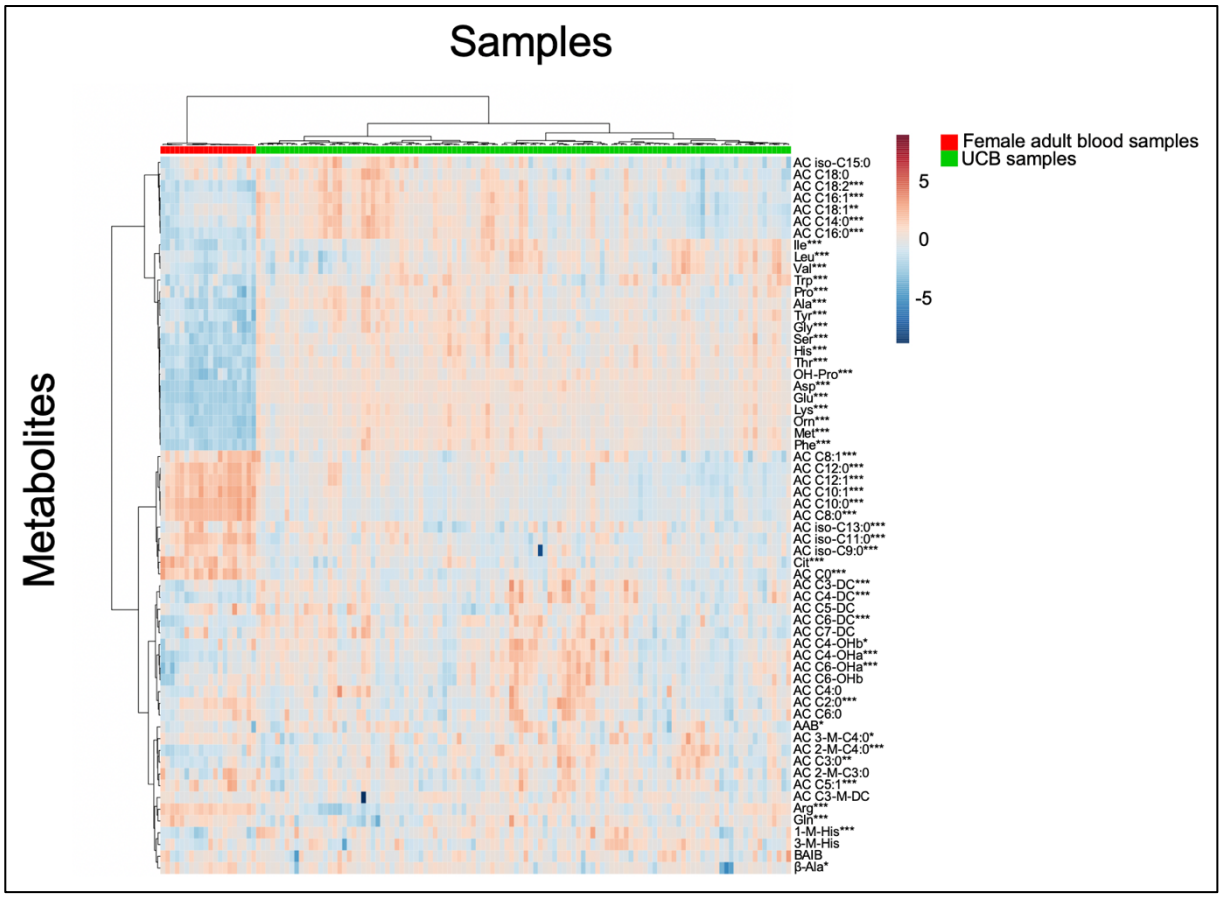

Figure 10: Heatmap of amino acids and acyl-carnitines in female adult blood (n = 20) and UCB of offspring of both sexes (n = 112) after hierarchical clustering. Analysis was performed using Spearman's rank correlation as similarity measure and the average linkage as agglomeration method. Concentrations are given in µmol/L. Red represents high, blue represents low concentrations. Statistical significance is based on p-values adjusted for multiple testing by the method of Benjamini-Hochberg. * = q-value < 0.05, ** = q-value < 0.01 *** = q-value < 0.001. A full list of metabolite abbreviations is provided in Table S 1. UCB, umbilical cord blood.

Hierarchical clustering clearly separated female adult blood from UCB, because UCB samples exhibited a greater variability than samples from women. The heatmap depicts two clusters of opposing metabolite concentrations in UCB and female adult blood. The cluster containing most amino acid i.e. isoleucine (Ile), leucine (Leu), valine (Val), tryptophan (Trp), proline (Pro), alanine (Ala), Tyr, Gly, Ser, histidine (His), threonine (Thr), 4-hydroxyproline (OH-Pro), Asp, Glu, lysine (Lys), Orn, Met, Phe showed higher concentration in UCB and lower concentrations in female adult blood. Another cluster showed lower concentrations in UCB and higher concentrations in female adult blood consisting of a group of medium-chain saturated and

monounsaturated acyl-carnitines, products of the β-oxidation: octanoylcarnitin (AC C8:0), octenoylcarnitine (AC C8:1), AC C10:0, decenoylcarnitine (AC C10:1), AC C12:0, and dodecenoylcarnitine (AC C12:1). A heatmap only depicting female adult blood vs. UCB of female offspring shows a different overall clustering (Figure S 2). However, the described subclusters of higher amino acid concentrations in UCB compared to female adult blood and lower concentrations of medium-chain saturated and monounsaturated acyl-carnitines in UCB compared to female adult blood were found here as well.

Multiple linear regression models were conducted to investigate whether known pre- or perinatal factors were accountable for the higher variability in UCB samples. Metabolite concentrations did not differ with regard to offspring sex, GA, mode of delivery, birth weight, GWG, and maternal HbA1c (%) at delivery when adjusted for multiple testing (Table S 4). Besides the batch effect, only mode of delivery and GA showed an impact on some of the metabolite concentrations before adjustment for multiple testing (Table 14). Offspring delivery by C-section had lower concentrations of Ala, Met, Orn, Phe, and Pro, whereas GA was inversely associated with OH-Pro concentration.

Table 14: Beta-estimate (95% CI) of amino acid concentrations in relation to pre- and perinatal factors significant before multiple testing

Metabolite	β-estimate (95% CI)	p-value
Mode of delivery (C-section vs. vaginal)		
Ala	-0.228 (-0.421; -0.036)	9.90E-03
Met	-0.194 (-0.3381; -0.0504	2.00E-03
Orn	-0.208 (-0.366; -0.051)	2.70E-03
Phe	-0.174 (-0.303; -0.044)	2.10E-03
Pro	-0.158 (-0.286; -0.030)	6.20E-03
GA		
OH-Pro	-0.121 (-0.179; -0.063)	<1.00E-03

Data are β-estimate (95% CI) and p-values of multiple linear regression models of n = 112 UCB samples with the categorical variables offspring sex (male/female), mode of delivery (C-section/vaginal), GWG excessive (excessive/ adequate), GWG inadequate (inadequate/ adequate), and high maternal HbA1c (%) at delivery (< 5.7 % vs. ≥ 5.7 %), and the continuous variables GA and birth weight as explanatory variables and the UCB metabolite, amino acids and acyl-carnitines, as outcome variable that were significant before multiple testing. Analysis was performed in n = 112 UCB samples. Offspring with missing data on any of the potential confounder were excluded from analysis. Metabolite concentrations are µmol/L. A full list of metabolite abbreviations is provided in Table S 1. CI, confidence interval; GA, gestational age; GWG, gestational weight gain, HbA1c, glycated hemoglobin

Another approach to assess differences or similarities between UCB and female adult blood was attempted via bi-clustering as hierarchical clustering approaches suffer from some drawbacks. In hierarchical clustering, each metabolite can only be part of one cluster, in turn, also each provided metabolite has to be part of a cluster. This procedure may not reflect the human physiology, since a metabolite could be part of several biological pathways, or might

only be identified in a specific subgroup or under specific circumstances. In contrast, bi-clustering simultaneously clusters rows and columns of a data matrix (194). This approach enables metabolites to be part of more than one cluster based on the similarity of metabolite concentrations. When analyzing female adult and UCB samples of both sexes, bi-clusters were only formed among adult blood samples, which confirmed higher similarities in adult samples compared to UCB samples (Figure 11).

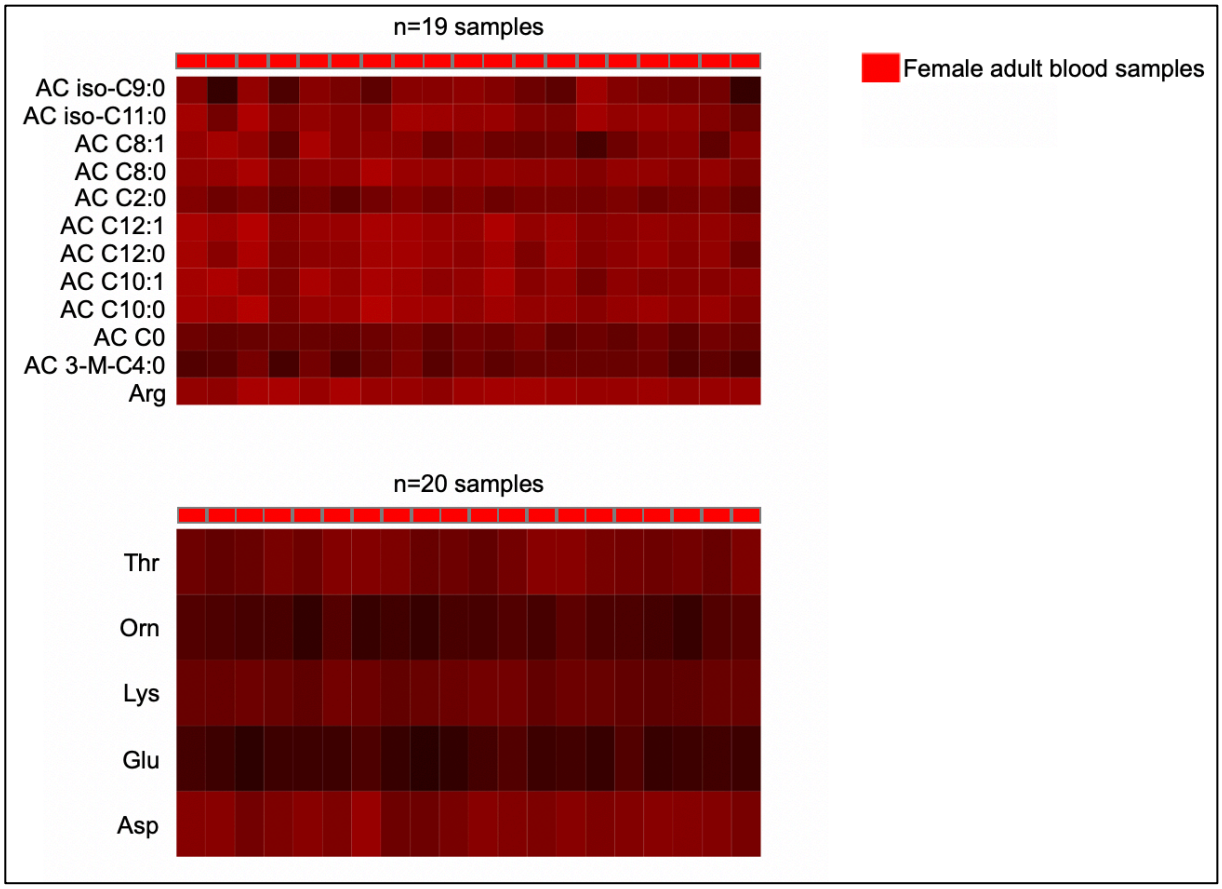

Figure 11: Bi-clustering of amino acids and acyl-carnitines based on female adult blood (n = 20) and UCB of both sexes (n = 112). Concentrations are given in µmol/L. A full list of metabolite abbreviations is provided in Table S 1.

Two clusters that entailed almost all adult blood samples were identified. The first bi-cluster mainly consisted of products of the β-oxidation i.e. AC C0, AC C2:0, AC C8:0, AC C8:1, AC C10:0, AC C10:1, AC C12:0, AC C12:1, a cluster that was also found in hierarchical clustering, thus confirming these findings. The second cluster consisted of the amino acids Thr, Orn, Lys, Glu, and Asp. Bi-clustering in female adult samples and female UCB samples resulted in only one clusters. This cluster was found among the female adult group and was similar to the first bi-cluster found when analyzing female adult and UCB samples of both sexes i.e. consisting of Arg, AC C0, AC C10:0, AC C10:1, AC C12:0, AC C12:1, AC C8:0, isoundecanoylcarnitine (AC iso-C11:0), isononanoylcarnitine (AC iso-C9:0) (Figure S 3).

3.2 Effect of prenatal factors on the UCB metabolite profile

For the main analysis, explorative multivariate analysis was conducted, which revealed that different classes of obesity seemed to have a greater impact on the UCB metabolite profile than a positive vs. negative GDM diagnosis in obese women (Figure 12). Thus, different degrees of maternal obesity were chosen as main exposure for subsequent analysis of the UCB metabolite profile.

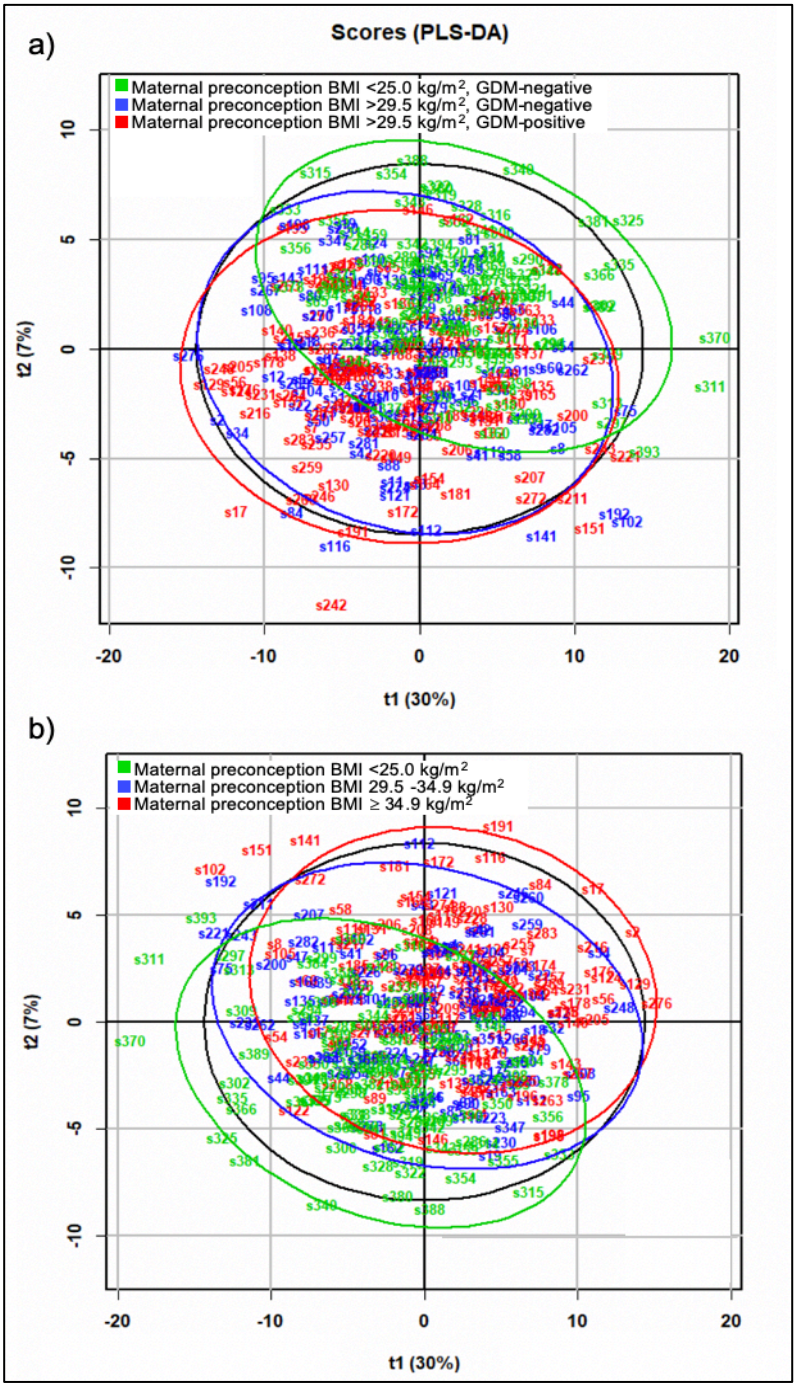

Figure 12: PLS-DA scores plots of UCB metabolite profiles of n = 398 samples according to a) maternal preconception obesity and GDM and b) different maternal obesity degrees. BMI, body mass index; GDM, gestational diabetes; PLS-DA projection to latent structures-discriminant analysis.

To gain an understanding of potential confounding factors, we depicted violin plots which show the variance of metabolite concentrations explained by several pre- and perinatal factors (Figure 13).

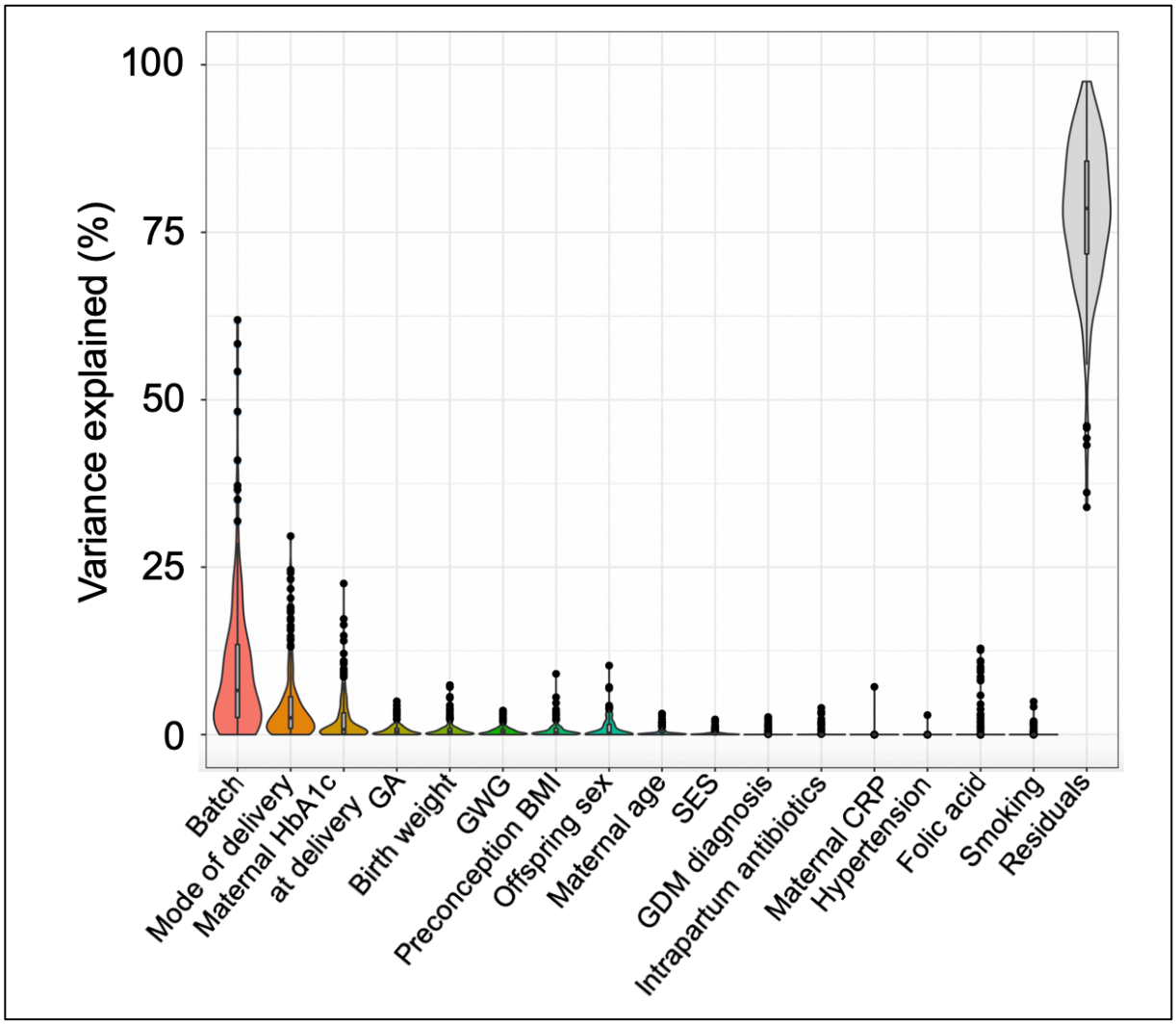

Figure 13: Violin plot depicting proportion of variance in metabolite concentrations of n = 398 UCB samples explained by pre- and perinatal factors. CRP, C-reactive protein; GA, gestational age; GDM, gestational diabetes mellitus; GWG, gestational weight gain; HbA1c, glycated hemoglobin; SES, socioeconomic status.

The largest proportion of factors explaining variance in metabolites remains unexplained by the presented pre- and perinatal factors. Batch effect makes up a large proportion of explainable variance though that factor is attributable to analytical rather than biological factors. Among the pre- and perinatal factors tested, mode of delivery and maternal HbA1c (%) at delivery had the largest effects followed by GA, birth weight, GWG, maternal preconception BMI and offspring sex. The heatmap in Figure 14 presents an overview of the influence of each factor on the variance of each metabolite concentration. Each field represents a metabolite. The trees of the dendrogram on the x-axis represent the similarity of the factors with regard to their influence on the variance of the metabolite concentration. In turn, the trees

on the y-axis represent the similarity of the variance of the metabolite concentrations in relation to the influence of the factors. The heatmap showed that each metabolite has a unique pattern i.e. is differently influenced by pre- and perinatal factors. Though the factors mode of delivery and GA seemed to be stronger in influencing metabolites, other metabolites are not affected by these factors at all. The clustering of metabolites, i.e. the similarity of the variance of the metabolite concentrations in relation to the influence of the factors, did not follow any obvious pattern such as metabolite class or metabolic pathways. This supports the common approach of using a set of factors for statistical analysis that explain the greatest variance possible for all metabolites alike.

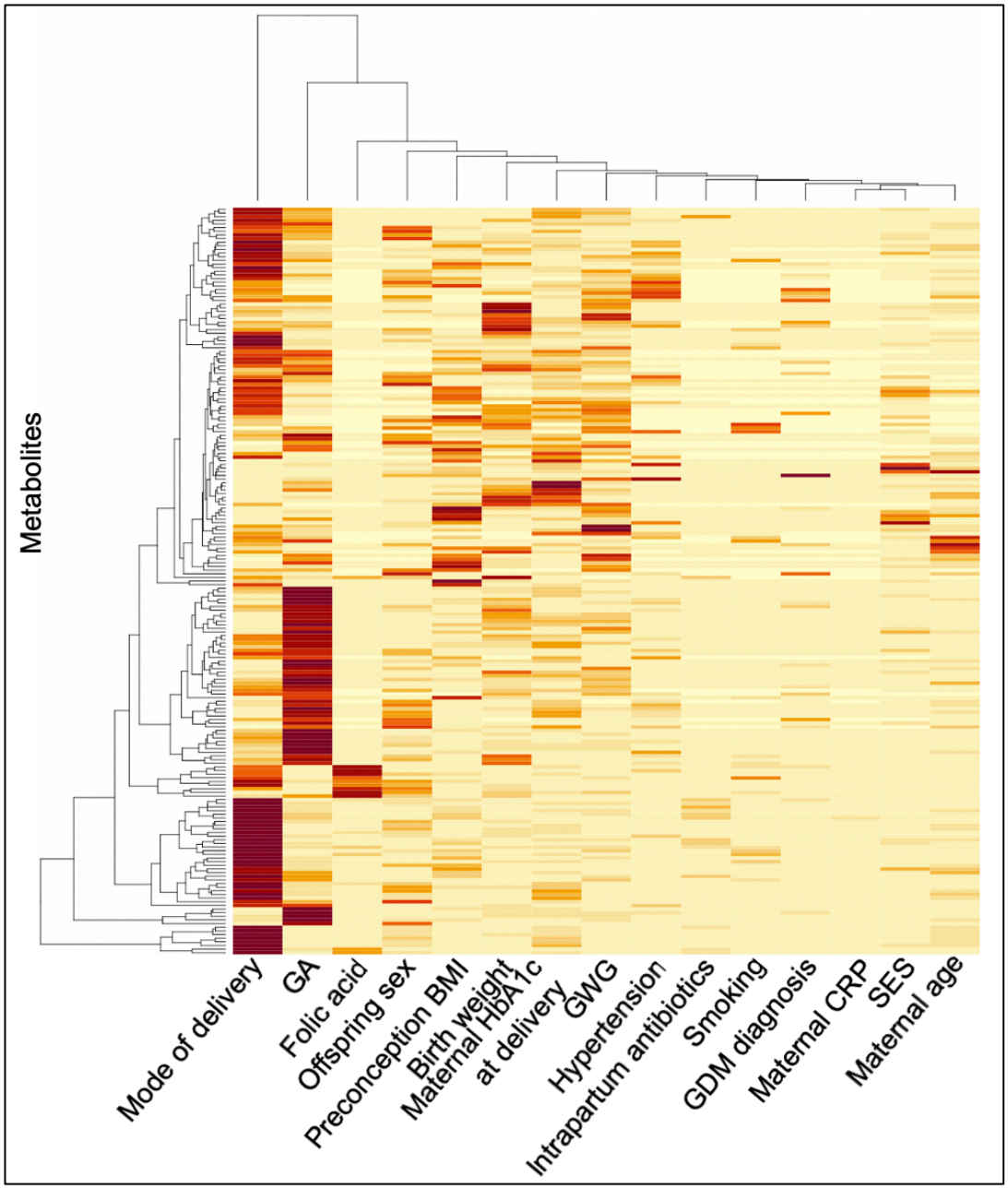

Figure 14: Heatmap depicting the influence of pre- and perinatal factors on the variance of UCB metabolite concentrations of n = 398 samples. Each field represents a metabolite, names were not

provided for reasons of visibility. Darker color indicates higher influence of the respective factor on variance of the metabolite concentration. CRP, C-reactive protein; GA, gestational age; GDM, gestational diabetes mellitus; GWG, gestational weight gain; SES, socioeconomic status.

3.3 Characteristics of the PEACHES study population

The study population comprised 398 offspring of preconception normal weight (n = 111), obese (n = 128) and severely obese (n = 159) mothers (Figure 15). The PEACHES dataset is prospectively growing. Time of data retrieval from the database of the main dataset: 23.06.2017. Data on offspring weight and height was last retrieved at 19.02.2019.

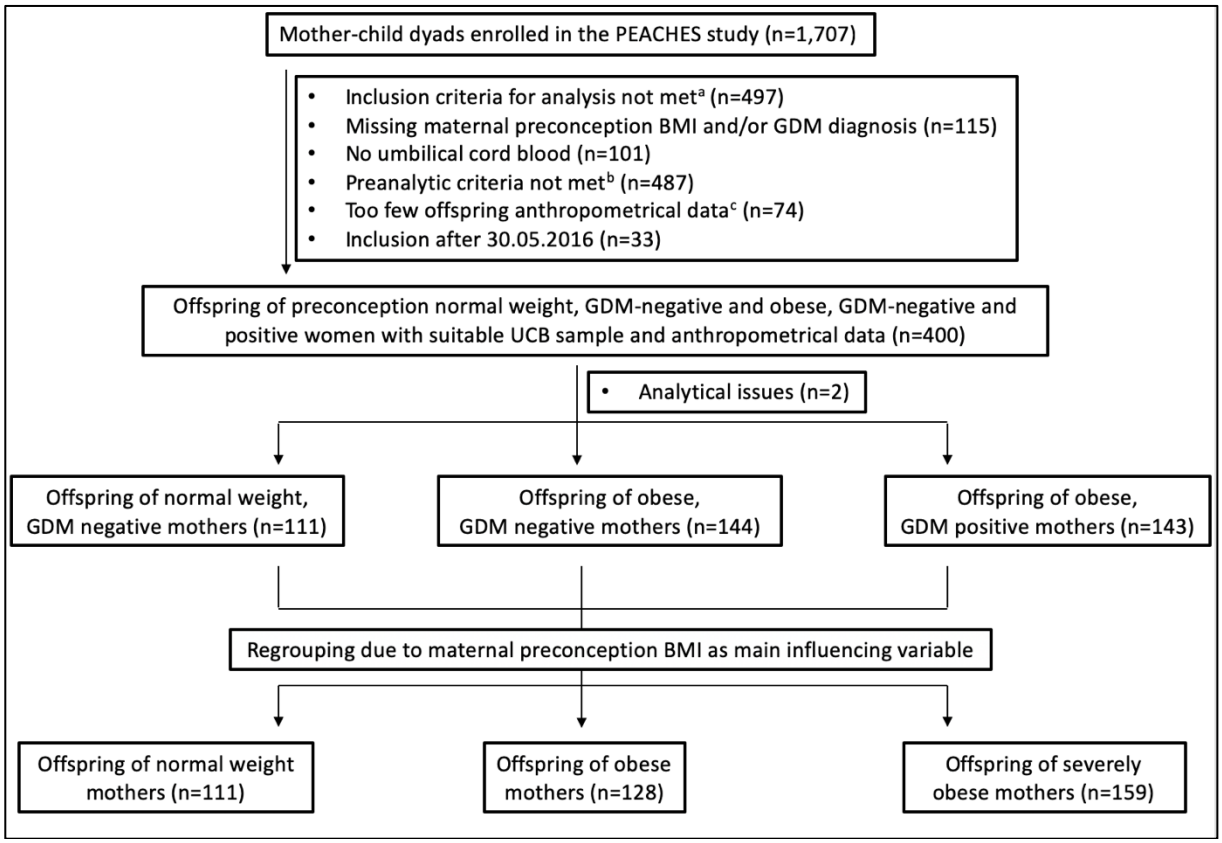

Figure 15: Flowchart sample selection [a]Inclusion criteria for analysis: singleton live birth, absence of type 1 diabetes and type 2 diabetes, maternal preconception normal weight and GDM-negative, maternal preconception obesity and GDM-positive or GDM-negative; [b]Pre-analytical criteria: non-hemolytic, time until centrifugation between 14 and 34 hours, adequate aliquot volume; or excluded for better alternative regarding aliquot availability, offspring sex, shorter time until centrifugation; [c]Too few offspring anthropometrical data due to later birth date or missing data; or excluded for better alternative regarding available anthropometrical data and offspring sex. GDM, gestational diabetes mellitus.

Characteristics of the study population are presented in Table 15. Maternal preconception BMI and GDM diagnosis differed between groups as per selection. 92.2 % of the maternal preconception BMIs were based on the first measured weight. The remaining maternal preconception BMIs were based on self-reported weight. Obese and severely obese mothers experienced more often maternal dysglycemia at delivery and had a higher C-section rate compared to normal weight mothers. Maternal HbA1c (%) at delivery, total GWG, continuous and categorical, breastfeeding and SES differed between all three groups. Maternal HbA1c

(%) at delivery was highest in severely obese women. Total continuous GWG was lowest in severely obese women. However, in contrast to normal weight women, where total GWG was within the adequate range, mean values of obese and severely obese women fell into the range classified as excessive GWG. Severely obese mothers where least likely to predominantly breastfeed longer or equal to one month and had the highest proportion of low SES. In relation to offspring of normal weight mothers, offspring of obese and severely obese mothers had slightly lower GAs and higher BMI z-scores at two and four years. Offspring of severely obese mothers were more often exposed to intrapartum antibiotics and had higher C-peptides when compared to those of normal weight mothers. The groups did not differ with regard to the maternal characteristics smoking during pregnancy, age at delivery, native language, and signs of maternal infection i.e. chorioamnionitis and CRP, or offspring sex, birthweight and offspring CRP.

Table 15: Maternal, perinatal and offspring characteristics of the PEACHES participants

Maternal/child characteristics		Offspring of normal weight mothers		Offspring of obese mothers		Offspring of severely obese mothers
	N		N		N	
Maternal and perinatal characteristics						
Preconception BMI, kg/m^2	111	22.0 (1.6)	128	**32.3 (1.6)**	159	**41.1 (5.0)***
Preconception BMI based on measured weight	111	103 (92.2)	128	116 (90.6)	159	148 (93.1)
GDM-positive	111	0 (0)	128	**56 (43.8)**	159	**87 (54.7)**
Late-pregnancy dysglycemia (HbA1c ≥ 5.7 %)	111	15 (13.5)	128	**39 (30.5)**	158	**66 (41.8)**
HbA1c at delivery, %[a]	111	5.3 (0.3)	128	**5.5 (0.4)**	158	**5.6 (0.4)***
Total GWG (continuous), kg	111	14.0 (4.9)	128	**11.4 (5.7)**	158	**9.7 (6.7)***
Total GWG (categorical), kg	111		128		158	
Adequate		49 (44.1)		**28 (21.9)**		**36 (22.8)***
Inadequate		30 (27.0)		**15 (11.7)**		**38 (24.1)***
Excessive		32 (28.8)		**85 (66.4)**		**84 (53.2)***
Smoking anytime during pregnancy	110	17 (15.5)	128	30 (23.4)	159	40 (25.2)
Maternal age at delivery	111	32.5 (4.7)	128	32.4 (5.0)	159	32.3 (5.3)
Native language (German)	111	84 (75.7)	128	107 (83.6)	159	126 (79.3)
Mode of delivery	111		128		159	
Vaginal		61 (55.0)		**60 (46.9)**		**57 (35.9)**
Vacuum-assisted		17 (15.3)		**8 (6.3)**		**5 (3.1)**
Elective C-section		17 (15.3)		**39 (30.5)**		**62 (39.0)**
Emergency C-section		16 (14.4)		**21 (16.4)**		**35 (22.0)**
Maternal CRP >15 mg/l	84	4 (4.8)	107	6 (5.6)	131	7 (5.3)
Chorioamnionitis	110	5 (4.6)	126	4 (3.1)	157	0 (0.0)
Intrapartum antibiotics	110	42 (38.2)	128	57 (45.2)	156	**86 (55.1)**
Offspring characteristics						
Sex: Female	111	60 (54.1)	128	65 (50.8)	159	80.0 (50.3)
GA, days	111	279.4 (8.8)	128	**274.6 (8.8)**	159	**274.1 (10.6)**
Birth weight, g	111	3447.0 (460.1)	128	3497.2 (400.2)	159	3525.7 (525.6)
Cord-blood C-peptide, ng/mL	110	0.58 (0.35)	128	0.66 (0.39)	159	**0.71 (0.41)**
Cord-blood CRP, mg/L	110	0.06 (0.51)	128	0.06 (0.62)	159	0.025 (0.32)

Breastfeeding (predominant ≥1 month)	111	86 (77.5)	128	**70 (54.7)**	159	**62 (39.0)***
SES at birth	107		126		151	
high		77 (72.0)		**59 (46.8)**		**37 (24.5)***
middle		27 (25.2)		**47 (37.3)**		**72 (47.7)***
low		3 (2.8)		**20 (15.9)**		**42 (27.8)***
BMI z-score at 1 years	111	0.0 (1.1)	128	0.3 (1.0)	159	**0.3 (1.0)**
BMI z-score at 2 years	109	0.4 (0.9)	128	**0.7 (0.9)**	152	**0.7 (1.1)**
BMI z-score at 3 years	106	0.2 (1.0)	120	0.5 (1.0)	148	0.5 (1.1)
BMI z-score at 4 years	99	0.1 (0.9)	109	**0.5 (1.0)**	126	**0.6 (1.2)**

Data are mean (SD) or n (%) and tested with regard to offspring of normal weight mothers using Student's t-test for continuous, parametric, Mann-Whitney-U for continuous, nonparametric and χ^2 tests for categorical variables. Bold fonts indicates p-values < 0.05 between offspring of normal weight and offspring of obese mothers, * indicates p-values < 0.05 between offspring of obese and offspring of severely obese mothers; [a]To convert HbA1c% to mmol/mol: IFCC HbA1c unit (mmol/mol) = [10.93 × DCCT/NGSP unit ± (%)] − 23.50; BMI, body mass index; CRP, C-reactive protein; C-peptide, connecting peptide; GA, gestational age; GDM, gestational diabetes; GWG, gestational weight gain; HbA1c, glycated hemoglobin; SES, socioeconomic status.

3.4 Effect of maternal preconception obesity on UCB metabolite profiles

OPLS regression was used to depict UCB metabolites according to maternal preconception BMI. OPLS regression is a supervised, multivariate analysis, which separately models the variations of the predictors, i.e. maternal preconception BMI, correlated and orthogonal to the response, i.e. UCB metabolites. The scores plot, where each dot represents the metabolite profile of one UCB sample, showed global deviations in samples obtained from offspring of normal weight, obese, and severely obese mothers (Figure 16).

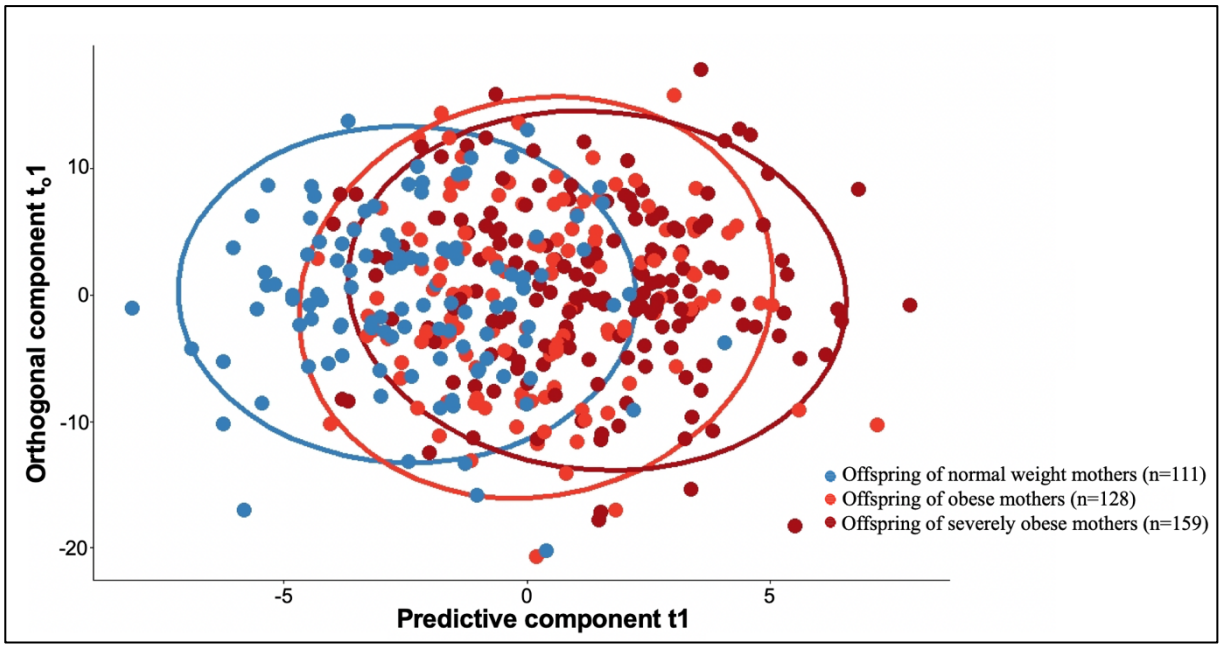

Figure 16: OPLS scores plot of UCB metabolite profiles (n = 398) according to maternal preconception BMI. BMI, body mass index; OPLS, orthogonal projection to latent structures.

The corresponding loading plot is shown four times, each time emphasizing another of the four analyzed metabolite groups for better clarity (Figure 17). Each dot represents a metabolite. The x-axis, the predictive component, of the loading plot presents the variation of metabolites between the three groups, i.e., which metabolites mainly contribute to differences in metabolite profiles of offspring of normal weight, obese and severely obese mothers. The further the dots are spread along the axis, the higher the variation. The metabolites furthest to left and right have the highest contribution. The y-axis, the orthogonal component, depicts the metabolite variations within each of the three groups, i.e. which metabolites contribute to different metabolite profiles within a group. As amino acids, acyl-carnitines, and bile acids are mainly distributed along the x-axis, concentrations of these metabolite groups are most likely contributing to variations between the three groups. Phospholipids are also distributed along the y-axis suggesting a contribution of the concentrations of phospholipids to inner group variations of the metabolite profiles.

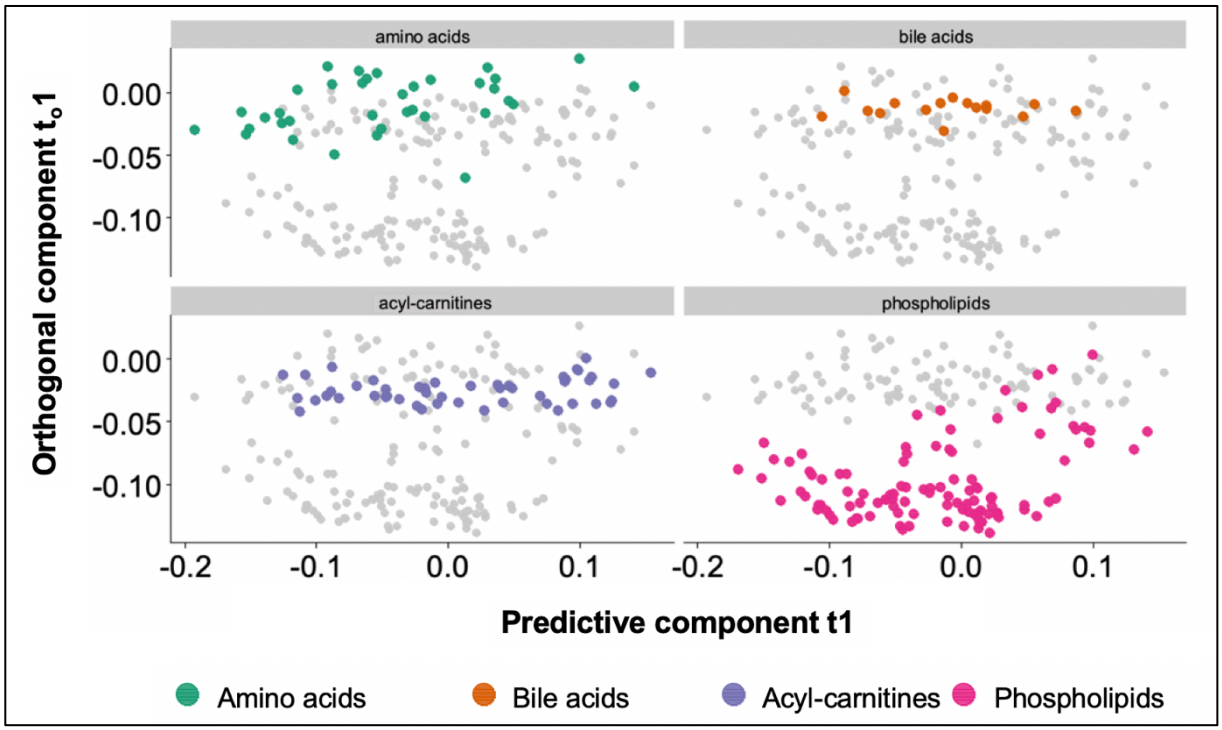

Figure 17: OPLS loading plot of UCB metabolite profiles (n = 398) according to maternal preconception BMI. BMI, body mass index; OPLS, orthogonal projection to latent structures.

Next, multiple linear regression models were used to compare the concentration of each metabolite, sums and ratios thereof and serum lipids in UCB of offspring of obese mothers (n = 128) and severely obese mothers (n = 159) to offspring of normal weight mothers (n = 111). β-estimates, standard error (SE), p- and q-values, are provided in the appendix (Table S 5). In addition, volcano plots were prepared to visualize alterations in metabolites (Figure 18). Therefore, log2 transformed UCB metabolite concentrations observed in offspring of obese

(Figure 18 a) and offspring of severely obese mothers (Figure 18 b) compared to offspring of normal weight mothers were expressed as fold changes (x-axis) and plotted against their -log transformed p-Value (y-axis). Metabolites that differed significantly after multiple testing based on the linear regression models were highlighted in red.

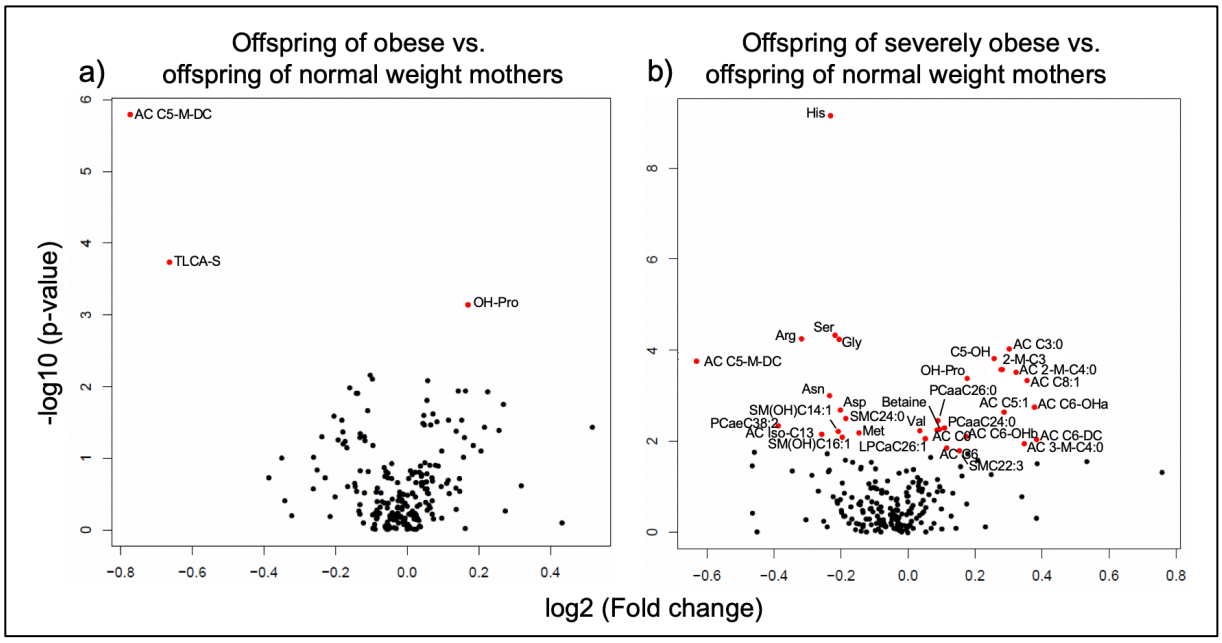

Figure 18: Volcano plots demonstrating alterations in UCB metabolites related to a) maternal obesity and b) maternal severe obesity. Metabolite concentrations were log2-transformed and expressed as fold changes of concentrations in offspring of normal weight mothers. Red points indicate q-value (p-value adjusted for multiple testing) < 0.05 based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders was excluded from the regression models. A full list of metabolite abbreviations is provided in Table S 1.

The quantity of metabolites whose concentrations differed significantly from those in offspring of normal weight mothers after multiple testing increased with the severity of obesity. Three metabolites, the acyl-carnitine AC C5-M-DC, the amino acids OH-Pro, and the secondary conjugated bile acid taurolithocholic acid-sulfate (TLCA-S), showed significantly different concentrations in UCB when comparing offspring of obese to offspring of normal weight mothers. In contrast, a total of 32 metabolites, the majority of which were amino acid and acyl-carnitines, showed significant alterations in offspring of severely obese mothers compared to offspring of normal weight mothers: the 14 acyl-carnitines AC C:0, AC C3:0, tiglylcarnitine and/or 3-methylcrotonylcarnitine (AC C5:1), AC C5-M-DC, AC C5-OH, hexanoylcarnitine (AC C6:0), adipylcarnitine (AC C6-DC), hydroxyhexanoylcarnitine L-isomere (AC C6-OHa), hydroxyhexanoylcarnitine D-isomere (AC C6-OHb), AC C8:1, isotridecanoylcarnitine (AC iso-C13), isobutyrylcarnitine (AC 2-M-C3:0), 2-methylbutyrylcarnitine (AC 2-M-C4:0), AC 3-M-C4:0; the ten amino acids Arg, Asn, Asp, betaine, Gly, His, Met, OH-Pro, Ser, Val; and the eight phospholipids LPC a C26:1, PC aa C24:0, PC aa C26:0, PC ae C38:2, SM(OH) C14:1,

SM(OH) C16:1, SM C22:3, SM C24:0. In addition, eight sums and ratios, which were not depicted in the volcano plots, showed significant differences in offspring of severely obese mothers after multiple testing.

Linear models were also applied after the dataset was stratified by offspring sex. β-estimates, standard error (SE), p- and q-values are provided in the appendix (Table S 6). The volcano plots in Figure 19 a-d depicts the fold changes of metabolite concentration when comparing male and female offspring of obese and severely obese mothers to male and female offspring of normal weight mothers, respectively.

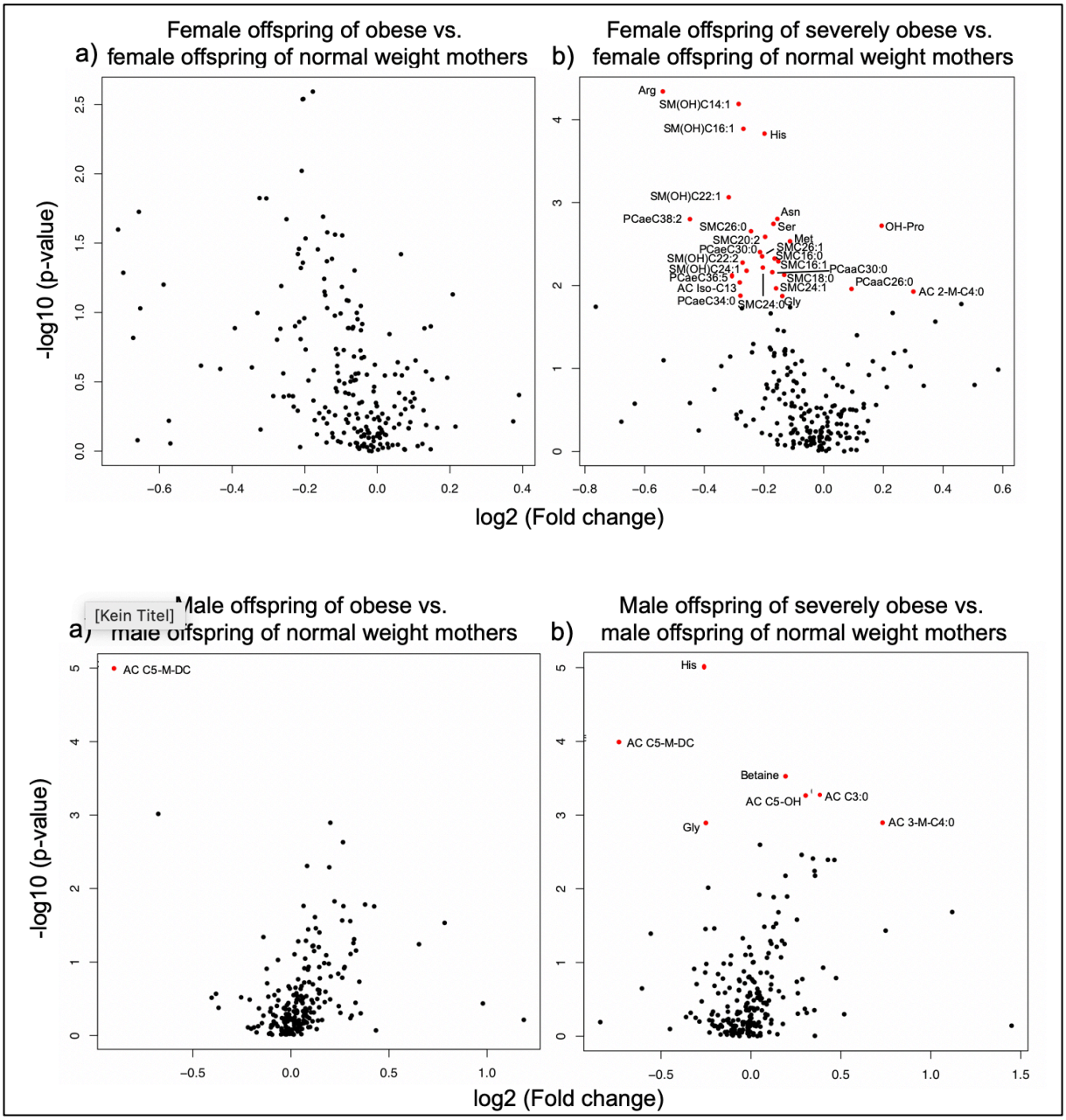

Figure 19: Volcano plots demonstrating alterations in UCB metabolites related to a) maternal obesity in female offspring, b) maternal severe obesity in female offspring, c) maternal obesity in male offspring, and d) maternal severe obesity in male offspring. Metabolite concentrations were

log2-transformed and expressed as fold changes of concentrations in offspring of normal weight mothers. Red points indicate q-value (p-value adjusted for multiple testing) < 0.05 based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 60 female and n = 51 male offspring of normal weight, n = 65 female and n = 63 male offspring of obese and n = 80 female and n = 79 male offspring of severely obese mothers after the dataset was stratified by offspring sex. Offspring with missing data on any of the potential confounders was excluded from the regression models. A full list of metabolite abbreviations is provided in Table S 1.

Stratification by sex revealed that maternal severe obesity altered more metabolites, especially phospholipids, in females than in males, and that alterations in 5 of 8 phospholipids detected in the entire cohort were driven by female offspring. No differences were detected between female offspring of obese and normal weight mothers after multiple testing. However, when comparing female offspring of severely obese those of normal weight mothers, the following 28 metabolites showed significant alterations: the 2 acyl-carnitines AC iso-C13:0, AC 2-M-C4:0; the 7 amino acids Arg, Asn, Gly, His, Met, OH-Pro, Ser; and the 19 phospholipids PC aa C26:0, PC aa C30:0, PC aa C36:5, PC ae C34:0, PC ae C30:0, PC ae C38:2, SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM (OH) C24:1, SM C16:0, SM C16:1, SM C18:0, SM C20:2, SM C24:0, SM C24:1, SM C26:0, SM C26:1. In addition, ten sums and ratios, which were not depicted in the volcano plot, showed significant differences between female offspring of severely obese mothers and those of normal weight mothers. After correction for multiple testing, AC C5-M-DC differed significantly when comparing male offspring of obese to those of normal weight mothers and the four acyl-carnitines AC 3-M-C4:0, AC C5-OH, AC C5-M-DC, AC C3:0 and three amino acids betaine, Gly, His when comparing male offspring of severely obese to male offspring of normal weight mothers. In addition, one ratio, which was not depicted in the volcano plots, showed significant differences in offspring of severely obese mothers after multiple testing.

When comparing the β-estimates of metabolite concentrations of male offspring of severely obese mothers to those of female offspring of severely obese mothers, significant differences were obtained between the sexes (Table S 7). We only considered β-estimates of metabolite concentrations which were associated with maternal severe obesity in at least one of the two sexes at least. Besides Arg and betaine, the metabolite profiles in female and male offspring of severely obese mothers mainly differed by SMs i.e. SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM C16:0, SM C18:0, SM C20:2, SM C26:0.

3.5 Effect of maternal preconception obesity on fetal BCAA catabolism

Among the 32 metabolites that differed significantly between UCB of offspring of severely obese and normal weight mothers after multiple testing, we focused on those that were connected via important metabolic pathways in the unstratified sample. One of these pathways was the BCAA degradation, in which Val, Leu and Ile are broken down delivering as final

products AC C2:0 and AC C3:0. Those can as acetyl-CoA and propionyl-CoA enter the tricarboxylic acid cycle for energy production or pathways of de novo fatty acid synthesis. An overview of the BCAA catabolism, including all metabolites and transcripts of gene encoding for enzymes involved in this pathway that were analyzed, is provided in Figure 20. Metabolites and transcripts that showed significant differences between offspring of severely obese and offspring of normal weight mothers were emphasized.

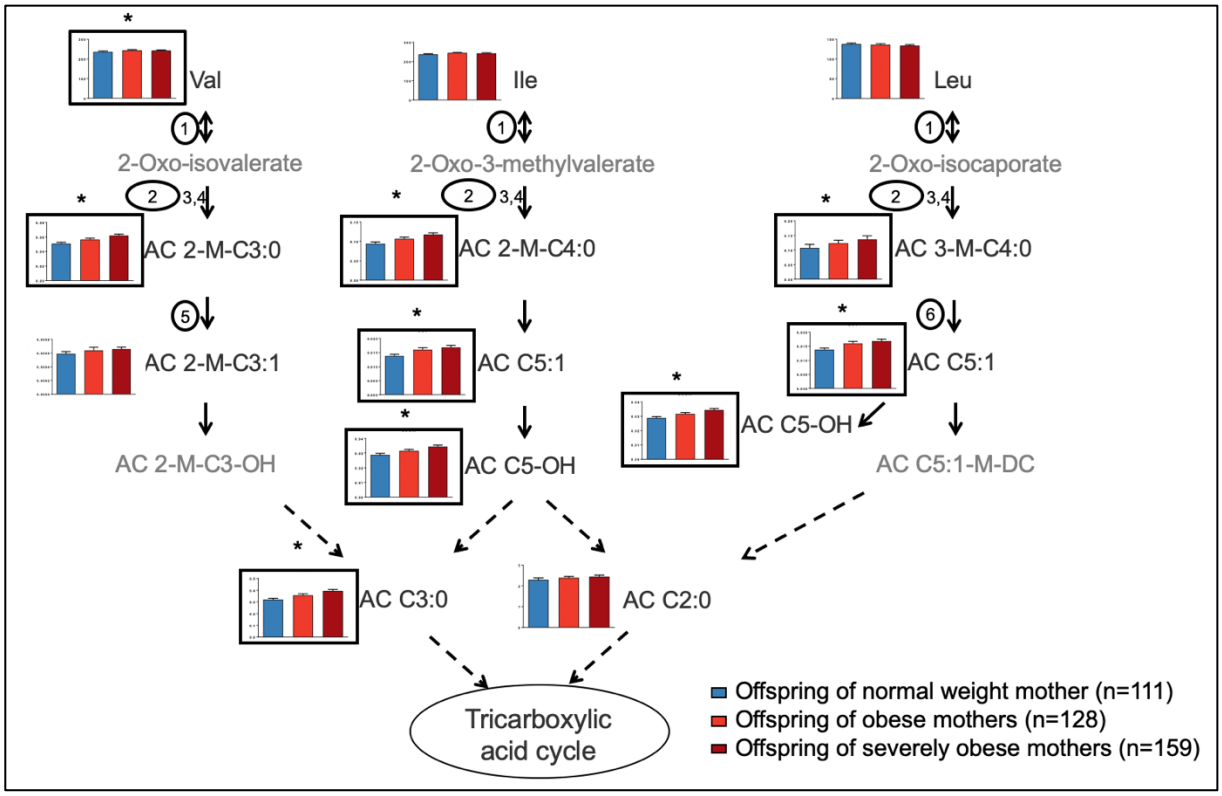

Figure 20: Alterations in the BCAA catabolism according to maternal preconception BMI. Barplots are given for all analyzed metabolites. Metabolite concentrations in UCB (µmol/L) are presented as barplots with mean + SEM of original concentrations in offspring of normal weight (n = 111), obese (n = 128) and severely obese (n = 159) mothers. Outliers above and below 4 SD of the mean were removed. * and bold frame indicate significant alteration (q-value (p-value adjusted for multiple testing) < 0.05) in metabolite concentration of offspring of severely obese compared to offspring of normal weight mothers based on linear regression models based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders was excluded from the regression models. Grey font indicates metabolites not analyzed within the pathway. Numbers indicate analyzed transcripts: 1=*BCAT1*, 2=*BCKDHA*, 3=*BCKDK*, 4=*DLD*, 5=*ACAD8*, 6=*IVD*. Bold circles indicate significantly different mRNA expression of genes (p-value < 0.05) in offspring of severely obese vs. normal weight mothers. A full list of metabolite abbreviations of analyzed metabolites is provided in Table S 1. AC 2-M-C3-OH, 3-hydroxyisovalerylcarnitine; AC C5:1-M-DC, 3-M-glutaconylcarnitine; BCAA, branched-chain amino acids, *BCAT1*, branched-chain amino acid transaminase 1; *BCKDHA*, branched chain keto acid dehydrogenase E1, alpha polypeptide; *BCKDK* branched chain ketoacid dehydrogenase kinase; *DLD*, dihydrolipoamide dehydrogenase; *IVD*, isovaleryl-CoA dehydrogenase; SEM, standard error of the mean. *Source: Figure adapted from* (172).

3.5.1 Metabolites

Val and the six metabolites of BCAA catabolism AC 2-M-C3:0, AC 2-M-C4:0, AC 3-M-C4:0, AC C5:1, AC C5-OH, and AC C3:0 were significantly higher in UCB of offspring of severely obese compared to offspring of normal weight mothers (Figure 20, Table 16).

Table 16: Associations between maternal preconception obesity and UCB BCAA-metabolites

	Offspring of obese vs. offspring of normal weight mothers			Offspring of severely obese vs. offspring of normal weight mothers		
Metabolite	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Val	0.067 (0.030)	2.57E-02	2.23E-01	0.086 (0.031)	5.84E-03	**2.46E-02**
AC 2-M-C3:0	0.159 (0.073)	2.98E-02	2.23E-01	0.280 (0.076)	2.68E-04	**3.56E-03**
AC 2-M-C4:0	0.143 (0.078)	6.76E-02	2.55E-01	0.296 (0.081)	3.11E-04	**3.71E-03**
AC 3-M-C4:0	0.241 (0.137)	7.89E-02	2.79E-01	0.362 (0.142)	1.14E-02	**3.78E-02**
AC C3:0	0.118 (0.071)	9.77E-02	3.21E-01	0.289 (0.074)	1.02E-04	**2.04E-03**
AC C5:1	0.162 (0.078)	3.70E-02	2.23E-01	0.248 (0.081)	2.26E-03	**1.42E-02**
AC C5-OH	0.129 (0.063)	4.25E-02	2.33E-01	0.250 (0.066)	1.62E-04	**2.62E-03**

Data are β-estimate (SE) of UCB metabolite concentrations (µmol/L) in offspring of obese and offspring of severely obese mothers in reference to offspring of normal weight mothers of significant findings within the BCAA-metabolism. Data are based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders was excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05. A full list of metabolite abbreviations was provided in Table S 1. BCAA, branched-chain amino acid; GA, gestational age; GWG, gestational weight gain; HbA1c, glycated hemoglobin; SE, standard error.

Offspring of obese mothers also showed a trend towards higher levels of the BCAA metabolites. These findings were not significant after adjusting for multiple testing.

3.5.2 Transcripts

To further assess alterations in BCAA catabolism, transcript levels of genes encoding for enzymes involved in the BCAA catabolism were analyzed in a subgroup.

3.5.2.1 Simultaneous isolation of RNA and DNA from PAXgene blood RNA tubes

As isolation of RNA and gDNA was necessary for subsequent analysis, the simultaneous isolation of RNA and gDNA from PAXgene Blood RNA tubes was established using control adult and UCB samples before the actual gene analysis in UCB samples. After isolation of gDNA and RNA from three UCB and six adult blood control samples, concentration and purity (260/280) and RNA integrity number (RIN) were analyzed (Table 17).

Table 17: gDNA and RNA concentration and quality markers of control samples

Sample	Sex	Concentration (ng/µL)	Yield µg	Purity (260/280)	Quality (RIN)
gDNA					
UCB1	Female	17.0	1.70	1.95	-
UCB2	Male	23.4	2.34	1.83	-
UCB3	Female	20.9	2.09	1.85	-

Adult 1	Male	15.8	1.6	1.92	-
Adult 2	Male	10.8	1.1	1.97	-
Adult 3	Female	14.3	1.4	2.15	-
Adult 4	Male	15.3	1.5	2.07	-
Adult 5	Female	16.1	1.6	2.12	-
Adult 6	Female	15.3	1.6	2.14	-
RNA					
UCB1	Female	173.4	13.87	2.11	7.1
UCB2	Male	463.3	37.06	2.04	8.6
UCB3	Female	443.5	35.48	2.08	9.2
Adult 1	Male	57.8	4.6	2.02	7.5
Adult 2	Male	89.4	7.2	2.05	7.8
Adult 3	Female	43.1	3.4	2.05	9.2
Adult 4	Male	68.0	5.4	2.13	7.3
Adult 5	Female	57.1	4.6	2.15	7.9
Adult 6	Female	79.4	6.3	2.15	7.6

gDNA, genomic deoxyribonucleic acid, RIN; RNA integrity number; RNA, Ribonucleic acid; UCB, umbilical cord blood

RNA concentration in UCB was five to six times higher compared to adult blood, due to an expectably higher cell count in UCB. gDNA had approximately 25% higher concentrations in UCB. Purity of RNA was >2, of gDNA >1.8 for both blood types.

To test functionality of gDNA and RNA, we amplified *ACADVL* in different quantities of gDNA and *IVD* in cDNA and separated samples using gel electrophoresis (Figure 21).

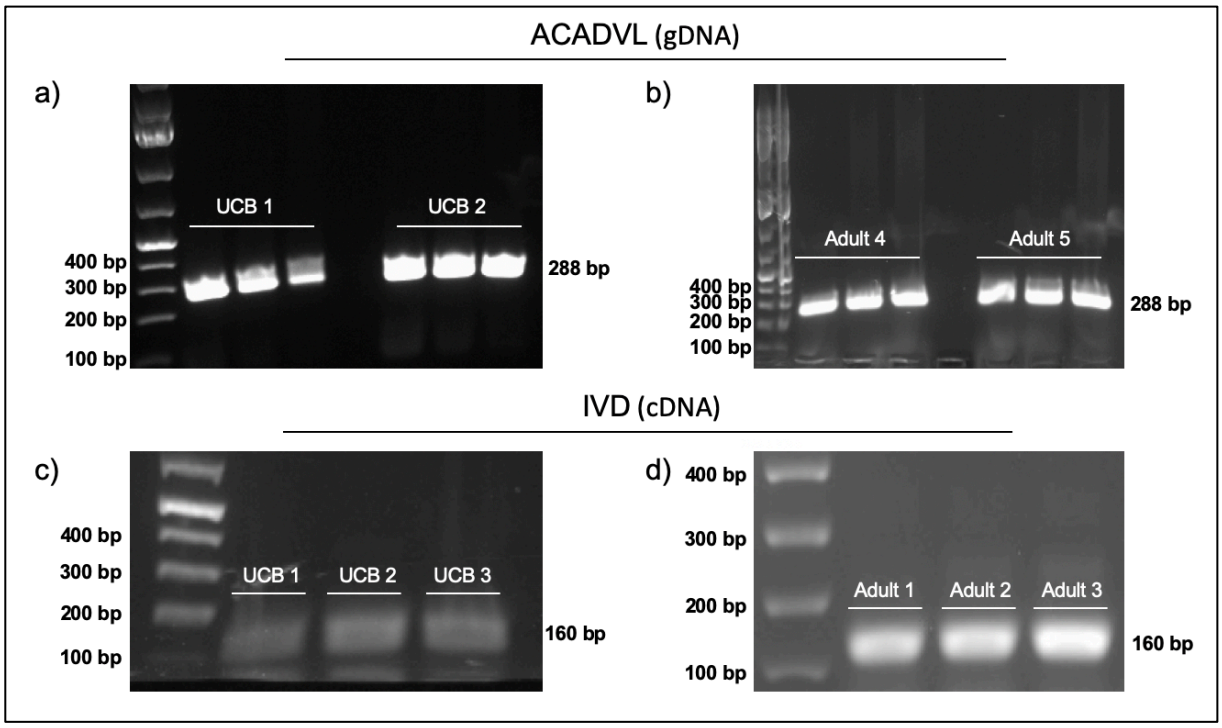

Figure 21: PCR and RT-PCR for verification of simultaneous isolation of RNA and DNA. ACADVL was amplified in gDNA via PCR and separated using 1% agarose gel in a) UCB and b) adult samples. IVD was amplified in cDNA via RT-PCR and separated using 1% agarose gel in c) UCB and d) adult

samples. ACADVL, acyl-CoA dehydrogenase very long chain; cDNA, complementary DNA; gDNA, genomic DNA; IVD, isovaleryl-CoA dehydrogenase; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; UCB, umbilical cord blood.

Both approaches provided positive results for the expected band size. No differences between UCB and adult blood were detected. Further, pyrosequencing was performed to test whether DNA of UCB and adult blood isolated from PAXgene is suitable to detect methylation patterns in promotor regions (Table 18).

Table 18: Pyrosequencing

Sample	Tubes	Methylation % Position 1	Methylation % Position 2
UCB1	PAXgene	91	70
UCB2	PAXgene	86	74
UCB3	PAXgene	86	67
Adult4	PAXgene	77	65
Adult	EDTA	82	61

UCB, umbilical cord blood.

gDNA isolated from PAXgenes showed the expected methylation patterns in the CpG isles in UCB and adult blood verifiying the suitability of these isolation products for methylation analysis. Thus, the simultaneous isolation of RNA and gDNA from PAXgene tubes is possible and results in nucleic acids of good quality which is feasible for molecular biological procedures.

3.5.2.2 Sample selection

Sample selection for gene expression analysis was based on maternal preconception BMI and percentiles of the six BCAA metabolic byproduct concentrations based on cumulative distribution curves as described in 2.6.1. In this subgroup, we defined “extremes” as offspring with the “highest” concentrations of the six BCAA metabolic byproducts in combination with highest maternal BMI and offspring with “normal” concentrations and normal maternal BMI. We selected the 17 samples from offspring of severely obese mothers (mean maternal preconception BMI was 40.9 ± 5.2 kg/m^2) that had the highest overall metabolite concentrations: at least four of the six BCAA byproducts had metabolite concentration of ≥90th percentile, the remaining metabolite concentrations were as high as possible i.e. ≥50th percentile. As controls, we selected 17 samples from offspring of normal weight mothers (mean maternal preconception BMI of 21.8 ± 1.8 kg/m^2) with metabolite concentrations <50th percentile and ≥10th percentile. When no more samples with metabolite concentrations in this category were left, we proceeded with samples of concentrations <10th percentile followed by those of <75th -≥50th percentile if former could not be achieved. Val was not considered for the selection process as its concentration showed no significant differences between offspring of

normal weight and offspring of severely obese mothers in initial regression models using a smaller set of potential confounders as in the final analysis (Table 16).

3.5.2.3 qRT-PCR

Based on preliminary analysis, we analyzed the following transcripts of genes encoding for enzymes that were related to the significantly altered metabolites of the BCAA catabolism: acyl-CoA dehydrogenase family member 8 (*ACAD8*), branched-chain amino acid transaminase 1 (*BCAT1*), branched chain keto acid dehydrogenase E1, alpha polypeptide (*BCKDHA*), branched chain ketoacid dehydrogenase kinase (*BCKDK*), dihydrolipoamide dehydrogenase (*DLD*), *IVD*. The relative mRNA expression levels of *ACAD8, BCAT1, BCKDHA, IVD* were significantly lower in offspring of severely obese compared to offspring of normal weight mothers (Figure 22).

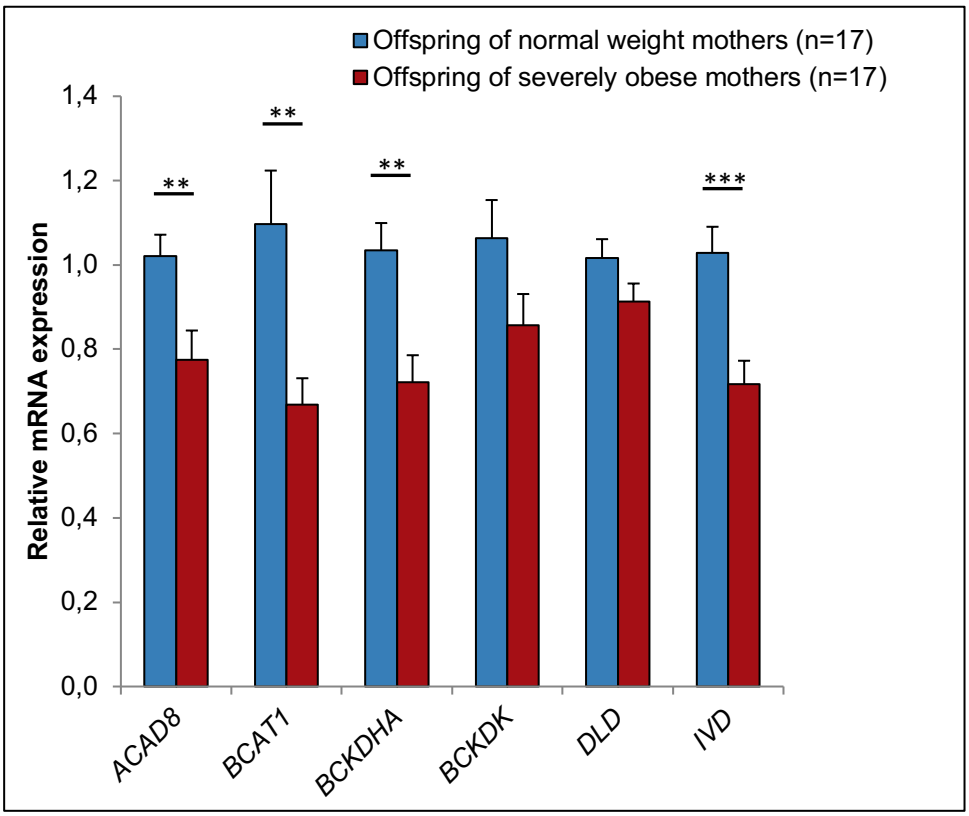

Figure 22: Alterations in gene expression involved in the branched-chain amino acid (BCAA) catabolism in UCB according to maternal preconception obesity. mRNA expression of *ACAD8, BCAT1, BCKDHA, BCKDK, DLD* and *IVD* in UCB of n = 17 offspring of normal weight vs. n = 17 offspring of severely obese mothers. Data are presented as mean + SEM. Significant differences were determined by two-tailed unpaired Student's t-test: ** p-value < 0.01, *** p-value < 0.001. *ACAD8*, acyl-CoA dehydrogenase family member 8. BCAA, branched-chain amino acid; *BCAT1*, branched-chain amino acid transaminase 1; *BCKDHA*, branched chain keto acid dehydrogenase E1, alpha polypeptide; *BCKDK* branched chain ketoacid dehydrogenase kinase; *DLD*, dihydrolipoamide dehydrogenase; *IVD*, isovaleryl-CoA dehydrogenase.

As shown in Figure 23 by scatterplots, the mRNA expression levels and metabolite concentrations were not correlated.

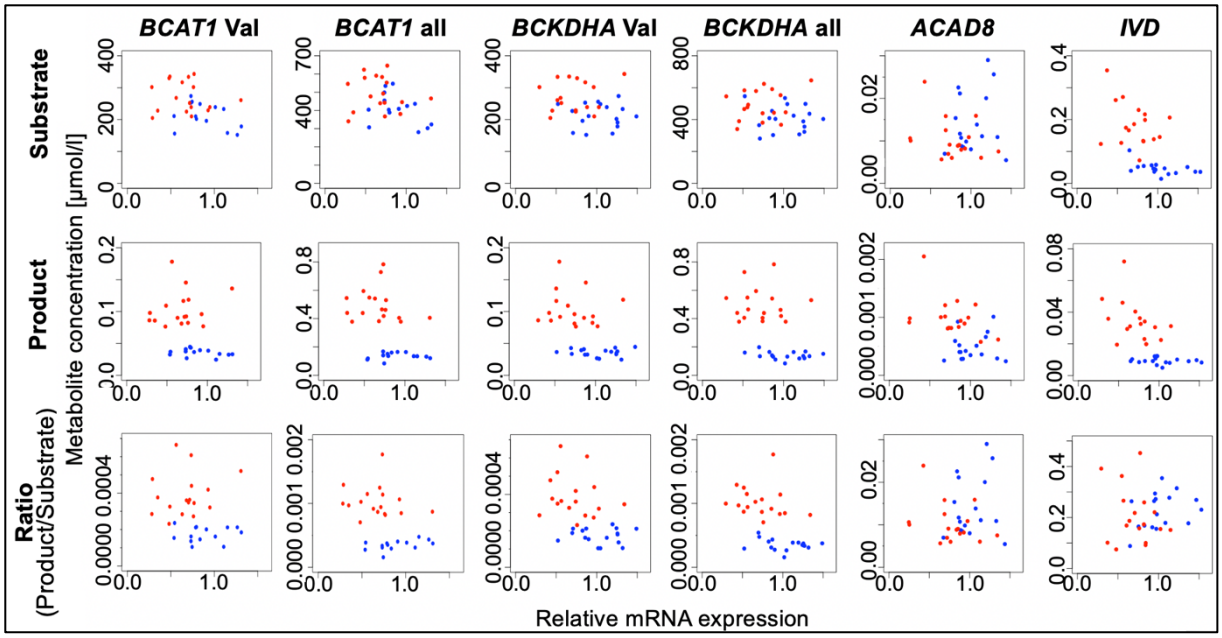

Figure 23: mRNA expression vs. metabolite concentrations of corresponding substrate, product, and ratio in UCB of offspring of normal weight and offspring of severely obese mothers. mRNA-Expression of *BCAT1* Val (Val vs. AC 2-M-C3:0), *BCAT1* all (sum of BCAAs vs. sum of products), *BCKDHA* Val (Val vs. AC 2-M-C3:0), *BCKDHA* all (sum of BCAAs vs. sum of products), *ACAD8* (AC 2-M-C3:0 vs. AC 2-M-C3:1), and *IVD* (AC 3-M-C4:0 vs. AC C5:1) is plotted against metabolite substrate, product and ratio concentration in UCB of n = 17 offspring of normal weight (blue dots) vs. n = 17 offspring of severely obese mothers (red dots). A full list of metabolite abbreviations was provided in Table S 1. *ACAD8*, acyl-CoA dehydrogenase family member 8; BCAA, branched-chain amino acid; *BCAT1*, branched chain amino acid transaminase 1; *BCKDHA*, branched chain keto acid dehydrogenase E1, alpha polypeptide; *IVD,* isovaleryl-CoA dehydrogenase; UCB, umbilical cord blood.

3.6 Effect of maternal preconception obesity on fetal one-carbon metabolism

The second pathway that was investigated in more detail was the one-carbon metabolism which integrates folate and methionine cycles that provide methyl groups for the synthesis of DNA and phospholipids. The one-carbon metabolism represented by all metabolites and transcripts of genes encoding for enzymes that were analyzed in this context is shown in Figure 24. Those that showed significant differences between offspring of severely obese and offspring of normal weight mothers were emphasized

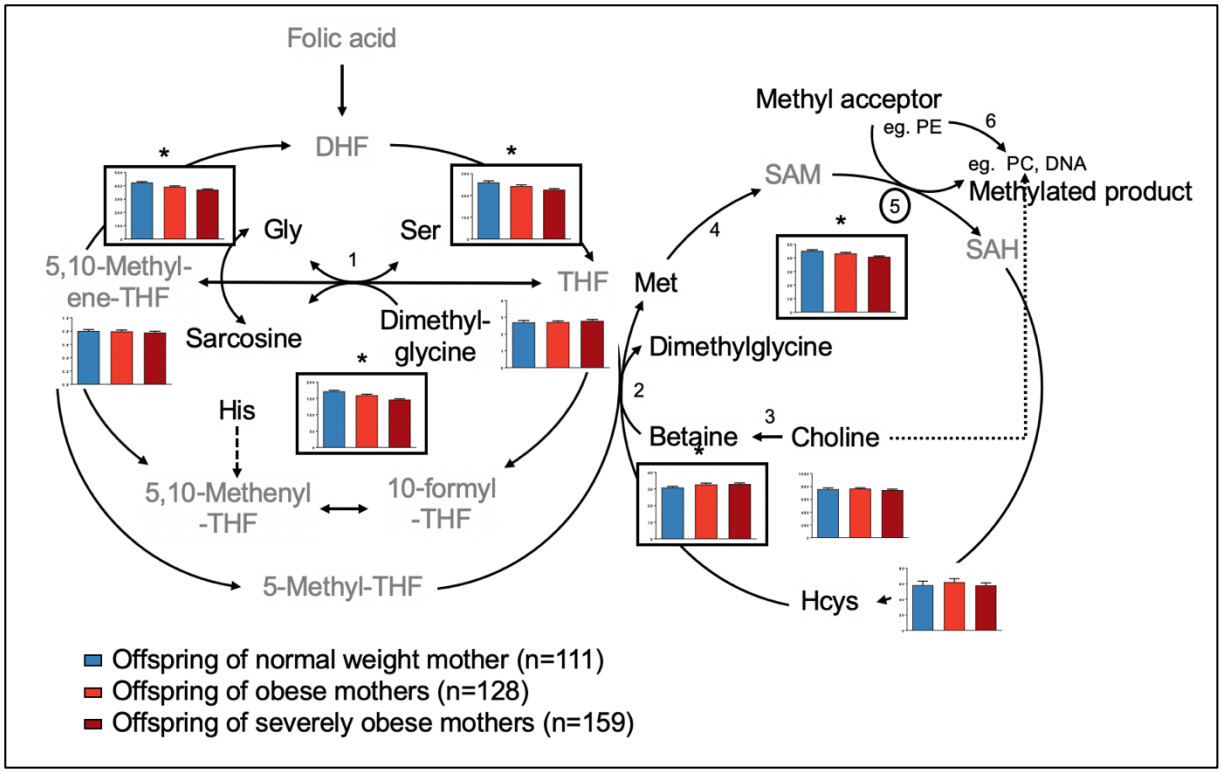

Figure 24: Alterations in the one-carbon metabolism in UCB according to maternal preconception obesity. Barplots are given for all analyzed metabolites. Metabolite concentrations (µmol/L) are presented as barplots with mean + SEM of original concentrations in offspring of normal weight (n = 111), obese (n = 128) and severely obese (n = 159) mothers. Outliers above and below 4 SD of the mean were removed. * and bold frames indicate significant alteration (q-value (p-value adjusted for multiple testing) < 0.05) in metabolite concentration of offspring of severely obese compared to offspring of normal weight mothers based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders was excluded from the regression models. Grey font indicates metabolites not analyzed within the pathway. Numbers indicate analyzed transcripts: 1=*SHMT1/2*, 2=*MTR*, 3=*ALDH7A1,* 4=*MAT2A*, 5=*DNMT1/3A/3B*, 6=*PEMT*. Bold circles indicate significantly different mRNA expression of genes (p-value < 0.05) in offspring of severely obese vs. normal weight mothers. A full list of metabolite abbreviations of analyzed metabolites is provided in Table S 1. *ALDH7A1*, aldehyde dehydrogenase 7 family member A1; DHF, dihydroxyfolate; *DNMT1/3A/3B*, DNA (cytosine-5)-methyltransferase 1/3A/3B; *MAT2A*, methionine adenosyltransferase 2A; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; PC, phosphatidylcholine; *PEMT*, phosphatidylethanolamine N-Methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SEM, standard error of the mean; *SHMT1/2*, serine hydroxymethyltransferase 1/2; THF, tetrahydrofolate. *Source: Figure adapted from* (195).

3.6.1 Metabolites

In the initial analysis, the amino acids Gly, His, Met, and Ser were found as significantly lower in UCB of offspring of severely obese compared to offspring of normal weight mothers (Figure 24, Table 19). Based on these findings, we aimed to subsequently analyze betaine, choline, dimethylglycine, sarcosine, and Hcys, five additional metabolites of the one-carbon metabolism. Of these, betaine showed significantly higher concentrations in UCB of offspring of severely obese compared to normal weight mothers.

Table 19: Associations between maternal preconception obesity and UCB one-carbon metabolism

	Offspring of obese vs. offspring of normal weight mothers			Offspring of severely obese vs. offspring of normal weight mothers		
Metabolite	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Betaine	0.100 (0.046)	3.09E-02	2.23E-01	0.135 (0.048)	5.54E-03	**2.46E-02**
Gly	-0.075 (0.033)	2.19E-02	2.23E-01	-0.138 (0.034)	5.64E-05	**1.35E-03**
His	-0.099 (0.037)	7.04E-03	1.96E-01	-0.241 (0.038)	7.04E-10	**8.42E-08**
Met	-0.041 (0.028)	1.55E-01	3.95E-01	-0.081 (0.030)	6.44E-03	**2.56E-02**
Ser	-0.105 (0.039)	7.88E-03	1.96E-01	-0.169 (0.041)	4.21E-05	**1.35E-03**

Data are β-estimate (SE) of UCB metabolite concentrations (µmol/L) in offspring of obese and offspring of severely obese mothers in reference to offspring of normal weight mothers of significant findings within the one-carbon metabolism. Data are based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05 A full list of metabolite abbreviations is provided in Table S 1. GA, gestational age; GWG, gestational weight gain; HbA1c, glycated hemoglobin; SE, standard error.

Offspring of obese mothers presented the same pattern. These findings were not significant after adjustment for multiple testing. Further, alterations in phosphatidylcholines, which are connected to the one-carbon metabolism were also observed in offspring of severely obese mothers i.e. higher concentrations in PC aa C24:0, PC aa C26:0, and lower concentrations in PC ae C38:2.

3.6.2 Transcripts

Analogously to BCAA catabolism, we profiled alterations in the one-carbon metabolism further by analyzing the mRNA expression of transcripts of genes encoding for enzymes involved in the pathway in a subgroup of “extremes”.

3.6.2.1 Sample selection

Sample selection was based on maternal preconception BMI and percentiles of the four amino acids Gly, His, Met, and Ser concentrations based on cumulative distribution curves as described in 2.6.1. In this subgroup, we defined “extremes” as offspring with “lowest” concentrations of the four amino acids in combination with highest maternal BMI and offspring with “normal” concentrations and normal maternal BMI. We selected the ten samples from offspring of severely obese mothers (mean maternal preconception BMI was 43.1 ± 6.1 kg/m^2) that had the lowest overall metabolite concentrations: at least two of four amino acids had metabolite concentration of <10th percentile, the remaining metabolite concentrations were <25th percentile. As controls, we selected ten samples from offspring of normal weight mothers (mean maternal preconception BMI of 22.0 ± 1.5 kg/m^2) that had metabolite concentrations <75th percentile and ≥25th percentile. As betaine was one of the metabolites subsequently

selected for analysis, it was analyzed after the selection process and could thus not be considered for transcription analysis.

3.6.2.2 qRT-PCR

Based on preliminary analysis, we analyzed the following transcripts of enzymes that were related to the significantly altered metabolites of the one carbon metabolism: aldehyde dehydrogenase 7 family member A1 (*ALDH7A1*), DNA methyltransferase 1 (*DNMT1*), DNA methyltransferase 3 alpha (*DNMT3A*), DNA methyltransferase 3 beta (*DNMT3B*), methionine adenosyltransferase 2A (*MAT2A*), 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*), *PEMT*, serine hydroxymethyltransferase 1 (*SHMT1*) and serine hydroxymethyltransferase 2 (*SHMT2*). The relative mRNA expression of *DNMT3A* was significantly lower in offspring of severely obese compared to offspring of normal weight mothers (Figure 25).

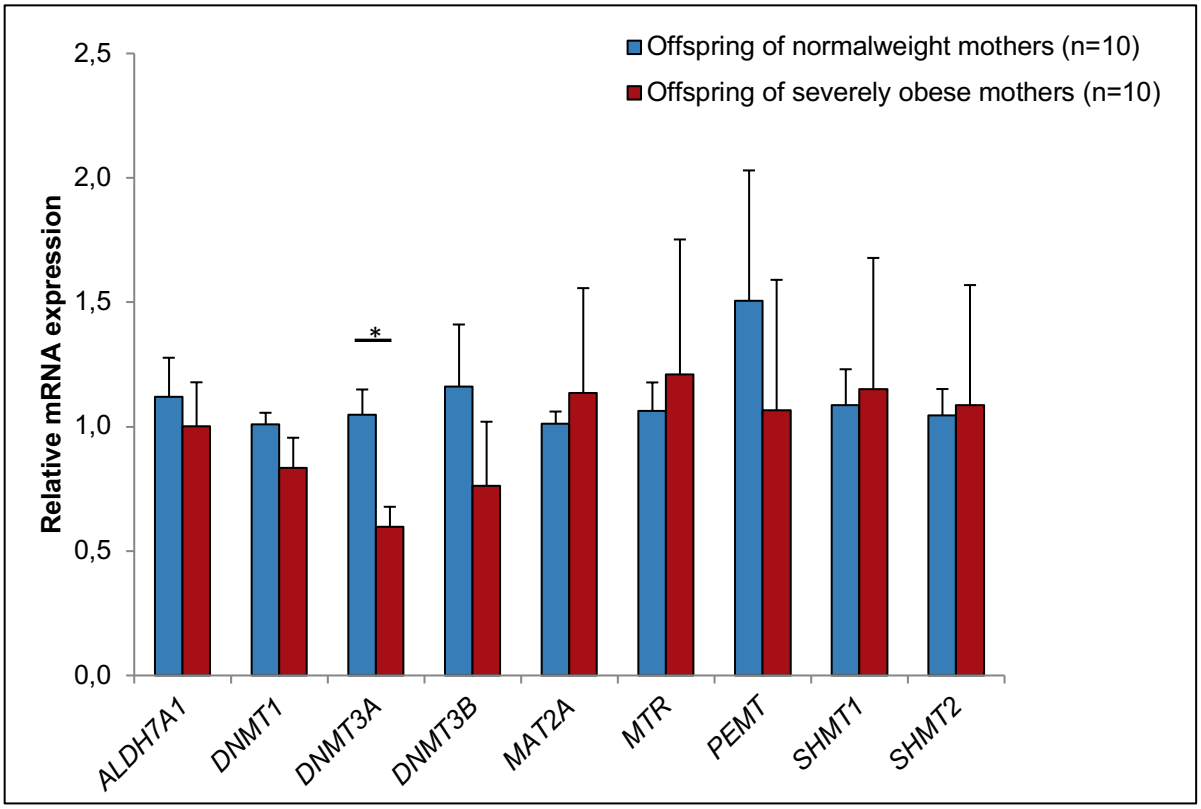

Figure 25: Alterations in gene expression involved in the one-carbon metabolism in UCB according to maternal preconception obesity. mRNA expression of *ALDH7A1, DNMT1, DNMT3A, DNMT3B, MAT2A, MTR, PEMT, SHMT1* and *SHMT2* in UCB of n = 10 offspring of normal-weight vs. n = 10 offspring of severely obese mothers. Data are presented as mean ± SEM. Significant differences were determined by two-tailed unpaired Student's t-test: * p-value < 0.05. *ALDH7A1*, aldehyde dehydrogenase 7 family member A1; *DNMT1/3A/3B*, DNA (cytosine-5)-methyltransferase 1/3A/3B; *MAT2A*, methionine adenosyltransferase 2A; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; *PEMT*, phosphatidylethanolamine N-Methyltransferase; SEM, standard error of the mean; *SHMT1/2*, serine hydroxymethyltransferase ½.

3.7 Effect of maternal late-pregnancy dysglycemia in obese women on UCB metabolite profiles

Based on our own preliminary work (153-155), we chose late-pregnancy dysglycemia within the group of obese mothers as an additional risk factor for adverse fetal outcome to investigate whether additional maternal metabolic complications further alter the fetal metabolite profile. We used multiple linear regression models to compare the concentration of each metabolite, sums and ratios thereof and serum lipids in UCB of offspring of obese mothers with high HbA1c at delivery (≥ 5.7 %; n = 106) to offspring of obese mothers with normal HbA1c at delivery (< 5.7 %; n = 181) (154). β-estimates, standard error (SE), p-value <0.05 were provided in the appendix (Table S 8). None of the metabolites remained significant after multiple testing. As this was an explorative analysis, we investigated associations with p-values < 0.05. Seven sums and ratios and the following 23 metabolites showed significant differences between offspring of obese mothers with a high HbA1c (%) at delivery and offspring of obese mothers with normal HbA1c (%) at delivery: malonylcarnitine (AC C3-DC), AC C8-OH, AC C12:0, AC C12:1, AC C14:0, AC C14:1, AC C15:0, AC C16:0, AC C16:1, AC C18:0, AC C18:1, octadecadienoylcarnitine (AC C18:2), Arg, AADP, GABA, Hcys, 1-Methylhistidine (1-M-His), glycodeoxycholic acid (GDCA), LysoPC a C14:0, LysoPC a C16:1, PC aa C42:0, PC aa C42:1, PC ae C40:6.

3.8 Effect of maternal late-pregnancy dysglycemia in obese women on UCB metabolites related to fetal β-oxidation

Among the 23 metabolites that differed (p-values < 0.05) between UCB of offspring of obese mothers with high and normal HbA1c (%) at delivery, saturated and monounsaturated medium- and long-chain acyl-carnitines were especially noticeable. Serum acyl-carnitines reflect the tissue pools of acyl-CoA (136). They have long been used as surrogates for impaired β-oxidation, as accumulation acyl-CoAs resulting from impaired β-oxidation cannot cross the mitochondrial membrane in contrast to the corresponding acyl-carnitines, which can be transported into the cytosol via the antiport SLC25A20 (136). Though the activity of SLC22A5, SLC25A20, and CPT2 have been proposed in the export of acyl-carnitines into the circulation, the actual transport mechanism remains unknown (141). An overview of the β-oxidation, including altered metabolites and all transcripts of genes encoding for enzymes that were analyzed in this context, is provided in Figure 26. Those that showed significant differences between offspring of obese and offspring of normal weight mothers were emphasized.

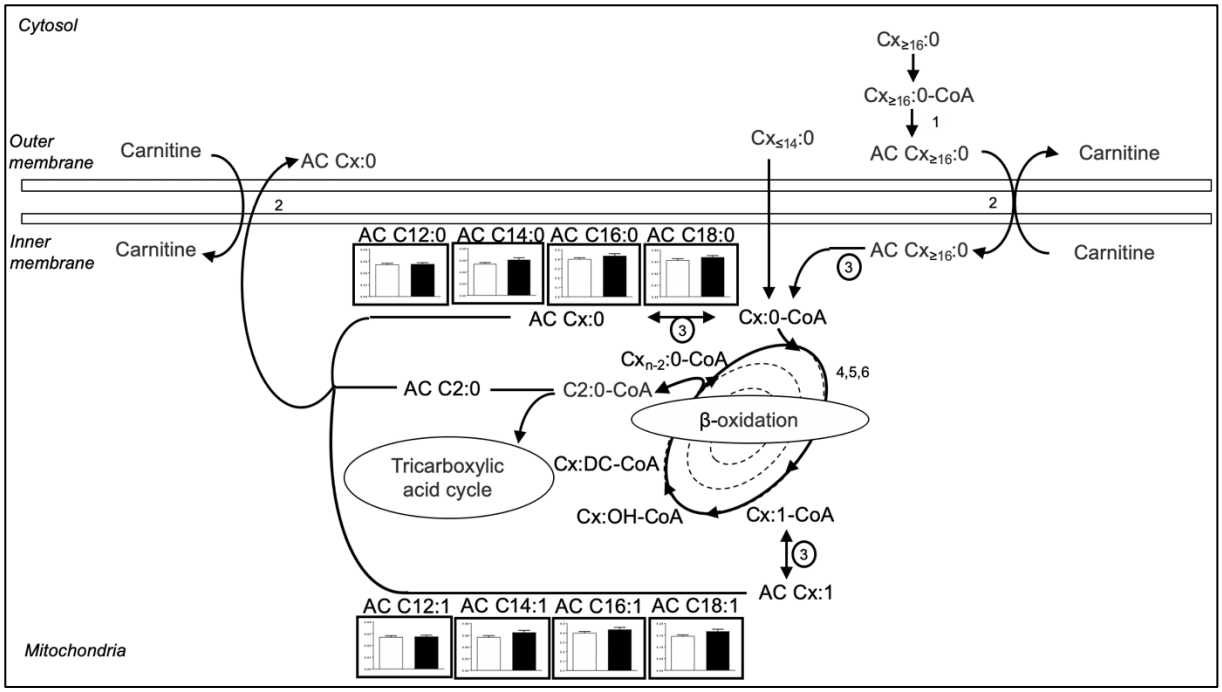

Figure 26: Alterations in the β-oxidation in UCB according to late-pregnancy dysglycemia in obese pregnancies. Barplots are given for all altered metabolites within the β-oxidation. Metabolite concentrations (µmol/L) are presented as barplots with mean + SEM of original concentrations in offspring obese mothers with normal (<5.7 %; n = 181) and high (≥ 5.7 %; n = 106) HbA1c at delivery. Outliers above and below 4 SD of the mean were removed. * and bold frames indicate significant alteration (p-value < 0.05) in metabolite concentration of offspring of obese with high HbA1c (%) at delivery compared to offspring of obese mothers with normal HbA1c (%) at delivery based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, and GWG performed in n = 106 offspring of obese mothers with high HbA1c at delivery and n = 181 offspring of obese mothers with normal HbA1c at delivery after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounders were excluded from the regression models.. Numbers indicate analyzed genes: 1=*CPT1A*/*B*, 2=*SLC25A20*, 3=*CPT2,* 4=*ACADVL*, 5=*ACADM*, 6=*ACADS*. Bold circles indicate significantly different mRNA expression of genes (p-value < 0.05) in offspring of obese mothers with high vs. normal HbA1c at delivery. A full list of metabolite abbreviations is provided in Table S 1. *ACADM,* acyl-CoA dehydrogenase medium chain; *ACADS,* acyl-CoA dehydrogenase short chain, *ACADVL,* acyl-CoA dehydrogenase very long chain; BMI, body mass index; *CPT1A/B/2* carnitine palmitoyltransferase 1A/1B/2; SEM, standard error of the mean; *SLC25A20*, solute carrier family 25 member 20. *Source: Figure adapted from* (196).

3.8.1 Metabolites

The eight medium- and long-chain acyl-carnitines AC C12:0, AC C12:1, AC C14:0, AC C14:1, AC C16:0, AC C16:1, AC C18:0, AC C18:1 were elevated in offspring of obese mothers with high HbA1c (%) compared to offspring of obese mothers with normal HbA1c (%) at delivery (Table 20).

Table 20: Associations between late-pregnancy dysglycemia in obese mothers and UCB β-oxidation

	Offspring of obese mothers with high HbA1c vs. offspring of obese mothers with normal HbA1c	
Metabolite	**β-estimate (SE)**	**p-Value**
AC C12:0	0.241 (0.096)	**1.29E-02**
AC C12:1	0.333 (0.094)	**4.58E-04**

AC C14:0	0.369 (0.110)	**9.19E-04**
AC C14:1	0.331 (0.106)	**2.08E-03**
AC C16:0	0.288 (0.108)	**7.95E-03**
AC C16:1	0.379 (0.114)	**9.60E-04**
AC C18:0	0.195 (0.092)	**3.57E-02**
AC C18:1	0.354 (0.104)	**7.60E-04**

Data are β-estimate (SE) of UCB metabolite concentrations (µmol/L) in offspring of obese (≥ 29.5 kg/m^2) mothers and high HbA1c at delivery (≥ 5.7 %) in reference to offspring of obese mothers with normal HbA1c at delivery (<5.7 %) of significant findings within the β-oxidation. Data are based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, and GWG performed in n = 106 offspring of obese mothers with high HbA1c at delivery and n = 181 offspring of obese mothers with normal HbA1c at delivery after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05. A full list of metabolite abbreviations is provided in Table S 1. BMI, body mass index; GA, gestational age; GWG, gestational weight gain; HbA1c, glycated hemoglobin; SE, standard error.

In addition, the ratio of (AC C16:0 + AC C18:0)/ AC C0 (product-to-substrate), which is related to the activity of CPT1 (185, 186), and the sum of medium- and long-chain acyl-carnitines were elevated in offspring of obese mothers with high HbA1c (%) at delivery.

3.8.2 Transcripts

We proceeded with confirmation of our data on another molecular level by analyzing mRNA expression of transcripts of genes encoding for enzymes involved in the β-oxidation in a small subgroup of extremes.

3.8.2.1 Sample selection

Sample selection was based on maternal HbA1c (%) at delivery in offspring of severely obese mothers and percentiles of the concentrations of the eight acyl-carnitines AC C12:0, C12:1, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and the ratio of (AC C16:0 + AC C18:0)/ AC C0 based on cumulative distribution curves as described in 2.6.1. In this subgroup, we defined “extremes” as offspring with “highest” concentrations of the nine acyl-carnitines in combination with high maternal HbA1c (%) at delivery and offspring with “normal” concentrations and normal maternal HbA1c (%) at delivery all in offspring of severely obese mothers. We selected the ten samples from offspring of severely obese mothers with a high HbA1c (%) at delivery (mean maternal preconception BMI was 40.8 ± 3.3kg/m^2) that had the highest overall metabolite concentrations: at least four of the nine acyl-carnitines had metabolite concentration of ≥90th percentile, the remaining metabolites were as high as possible i.e. the majority ≥50th percentile. As controls, we selected ten samples from offspring of severely obese mothers with normal HbA1c (%) at delivery (mean maternal preconception BMI of 39.0 ± 3.1 kg/m^2) that had metabolite concentrations <50th percentile and ≥10th percentile in eight of nine metabolites. When no more samples with metabolite concentrations in this category were left, we

proceeded with samples of concentrations <10th percentile followed by those of <75th -≥50th percentile if former could not be achieved.

3.8.2.2 qRT-PCR

We analyzed the following transcripts of enzymes that were related to the significantly ($p \leq 0.05$) altered metabolites of the β-oxidation: acyl-CoA dehydrogenase medium chain (*ACADM*), acyl-CoA dehydrogenase short chain (*ACADS*), *ACADVL*, *CPT1A*, *CPT1B*, *CPT2*, *SLC25A20*. The relative mRNA expression levels of *CPT2* was significantly lower in offspring of severely obese mothers with high HbA1c (%) at delivery compared to offspring of severely obese mothers with normal HbA1c (%) at delivery (Figure 27).

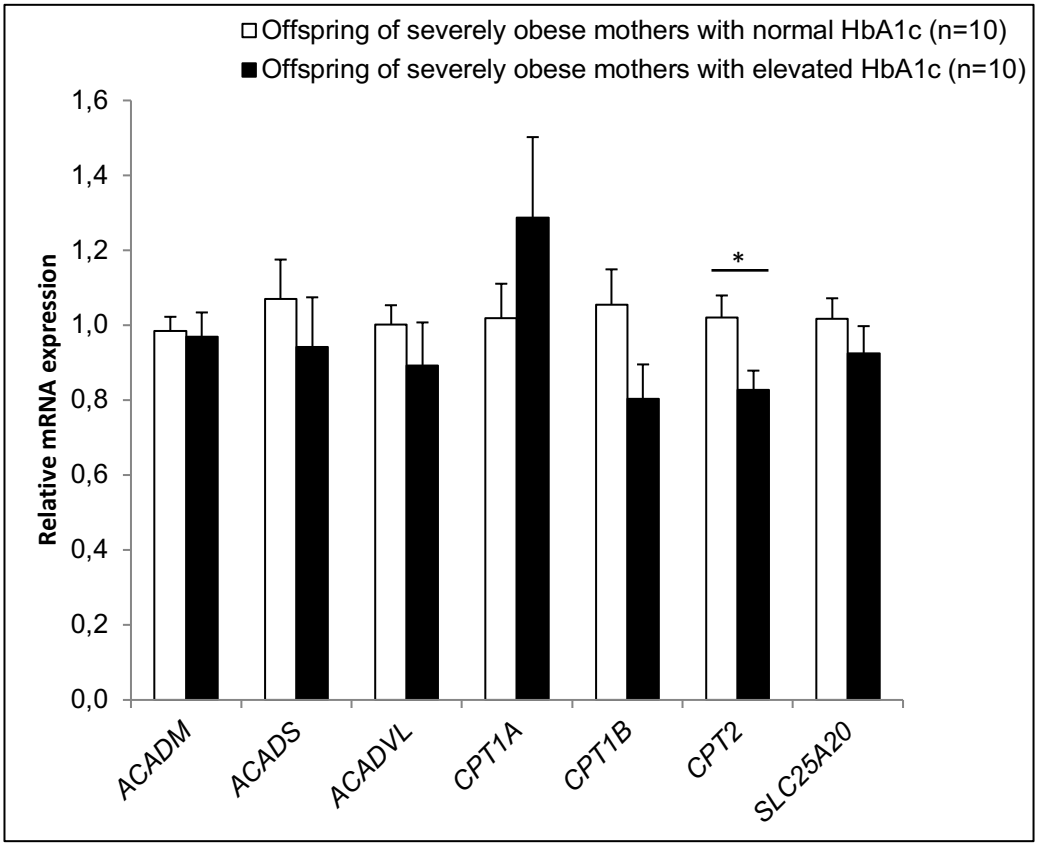

Figure 27: Alterations in genes involved in the β-oxidation in UCB according to late-pregnancy dysglycemia in obese women. mRNA expression of *ACADM, ACADS, ACADVL, CPT1A, CPT1B, CPT2,* and *SLC25A20* in UCB of n = 10 offspring of obese mothers with normal HbA1c at delivery (<5.7 %) vs. n = 10 offspring of obese mothers with high HbA1c at delivery (≥ 5.7 %). Data are presented as mean ± SEM. Significant differences were determined by two-tailed unpaired Student's t-test: * p-value < 0.05. *ACADM,* acyl-CoA dehydrogenase medium chain; *ACADS,* acyl-CoA dehydrogenase short chain, *ACADVL,* acyl-CoA dehydrogenase very long chain; *CPT1A,* carnitine palmitoyltransferase 1A; *CPT1B,* carnitine palmitoyltransferase 1B; *CPT2,* carnitine palmitoyltransferase 2; SEM, standard error of the mean; *SLC25A20*, solute carrier family 25 member 20.

3.9 Global Methylation of UCB DNA in offspring of severely obese mothers

Analysis of UCB metabolites suggested alterations in the one-carbon metabolism caused by severe maternal preconception obesity. The one-carbon metabolism is linked to DNA methylation, an epigenetic process (88). Thus, we analyzed global methylation of UCB DNA in offspring of n = 20 severely obese and those of n = 20 normal weight mothers via the mean UCB content of 5-mC to investigate a possible impact of maternal severe obesity on epigenetic mechanism. Three samples of offspring of severely obese and two samples of offspring of normal weight mothers had to be excluded from the analysis as they were outside the linear range of the standard curve (Figure 28).

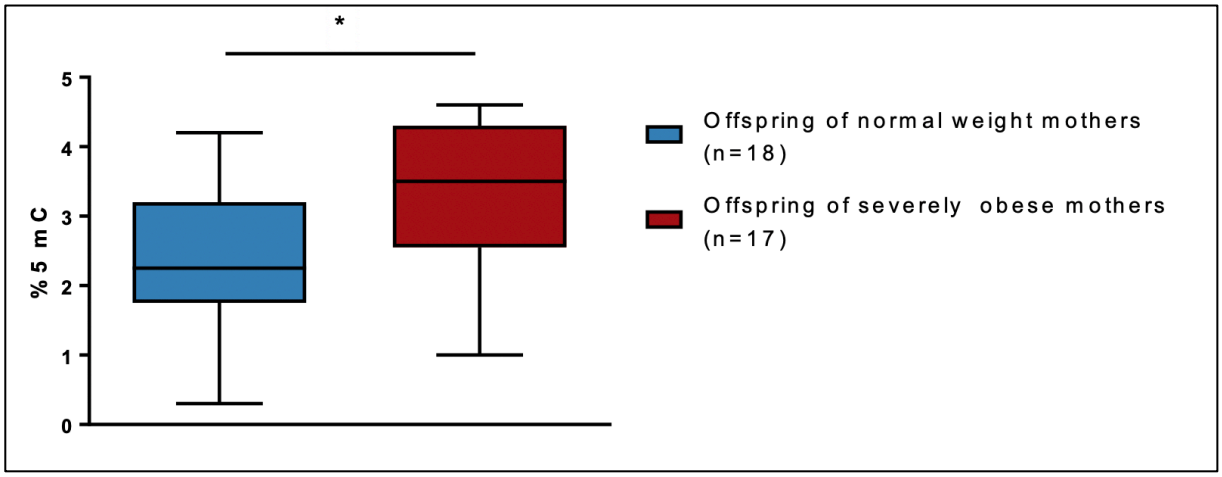

Figure 28: Impact of severe maternal preconception obesity on global methylation in UCB. %5mC of UCB was analyzed in n = 18 samples of offspring of normal weight vs. n = 17 samples offspring of severely obese mothers. Data are presented as median (horizontal lines within the boxes), 25th and 75th centile (lower and upper boundaries of the boxes), and 1.5 times the interquartile range (whisker ends). Significant differences were determined by two-tailed unpaired Student's t-test: * p-value < 0.05. UCB, umbilical cord blood.

A mean content of 5-mC of 3.3 ± 1.1% was detected in the offspring of severely obese mothers (mean maternal preconception BMI: 41.0 ± 7.3 kg/m^2) and 2.4 ± 1% in the group of offspring of normal weight mothers (mean maternal preconception BMI: 22.5 ± 1.6 kg/m^2). Thus, global DNA methylation in offspring of severely obese mothers was significantly increased in average by approximately 38%.

3.10 UCB metabolites and offspring BMI outcome

In this section, the relationship between UCB metabolites and preschool weight outcome in offspring of normal weight and offspring of obese, i.e. all offspring of mothers with a preconception BMI ≥ 29.5 kg/m^2, was investigated. Therefore, the longitudinal weight development and BMI z-scores at fixed time points were analyzed.

3.10.1 Offspring longitudinal BMI development during preschool age

First, we compared the longitudinal BMI development in offspring of age one to four years from obese (n = 287) and normal weight mothers (n = 111) (Figure 29). The longitudinal development i.e. the BMI z-score slope reflecting the change of BMI z-scores from age one to four years was significantly ($p < 0.007$) higher in offspring of obese mothers.

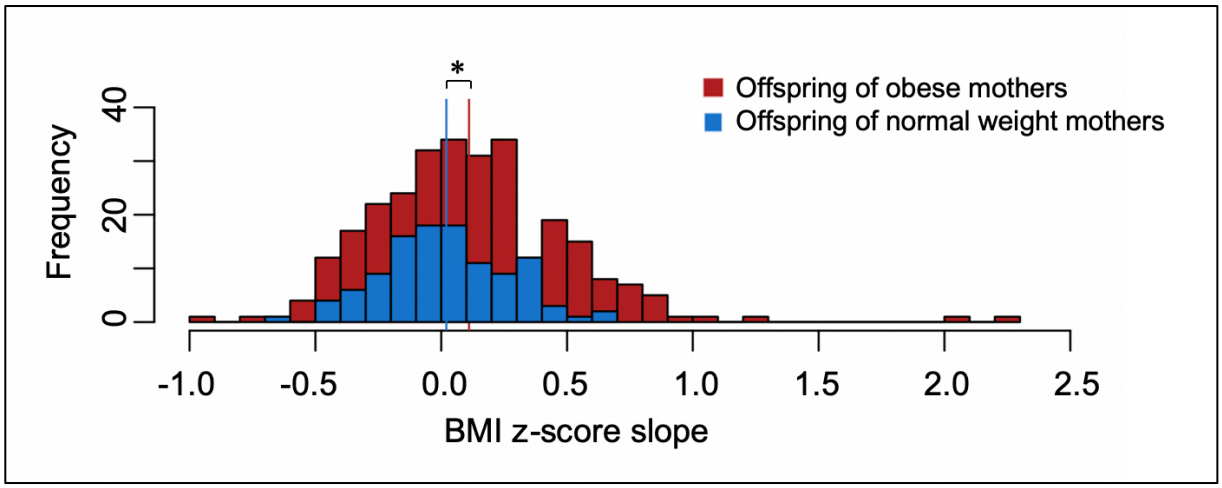

Figure 29: Histogram of BMI z-score slopes in offspring of age one to four years from obese and those of normal weight mothers. BMI z-scores were calculated based on WHO Child Growth Standards (165). BMI z-score slopes i.e. the change of BMI z-score from year one to four years were estimated using a linear regression model for each child (n = 398) based on respective BMI z-scores. BMI, body mass index; WHO, World Health Organization.

3.10.2 Effect of metabolites in UCB on the offspring preschool BMI z-score slope

We next investigated the relationship between the longitudinal BMI development, i.e. the BMI z-score slope, from age one to four years and UCB metabolites in offspring of obese (n = 287) and those of normal weight mothers (n = 111) (Table S 9). As none of the associations remained statistically significant after correction for multiple testing, we conducted sensitivity analysis to test associations (p-values < 0.05) by further adjusting models for maternal preconception BMI and maternal HbA1c (%) at delivery, and by using metabolite residuals (Table S 10), the value after correcting the metabolite log2-transformed concentrations for potential confounding factors used to investigate association between UCB metabolites and maternal obesity (Table S 5). Most of the associations were stable throughout sensitivity analyses indicating that findings were not simply due to chance. The associations of metabolite concentrations with the BMI z-score slope from age one to four years (p-values < 0.05) in offspring of obese and those of normal weight mothers were depicted as forest plots in all, male and female offspring of obese and normal weight mothers (Figure 30). Corresponding β-estimates were provided in the appendix (Table S 11).

The metabolites associated with the BMI z-score slope from one to four years of age in offspring of obese mothers differed from the one in offspring of normal weight mothers. In offspring of obese mothers, a higher BMI z-score slope from age one to four years was mainly inversely associated with UCB metabolites of lower concentrations including the amino acids Leu and choline, the acyl-carnitines AC 3-M-C4:0 and AC C4:1, PC aa 38:4, and LDL cholesterol. Positive associations were found with betaine and AC C7:0.

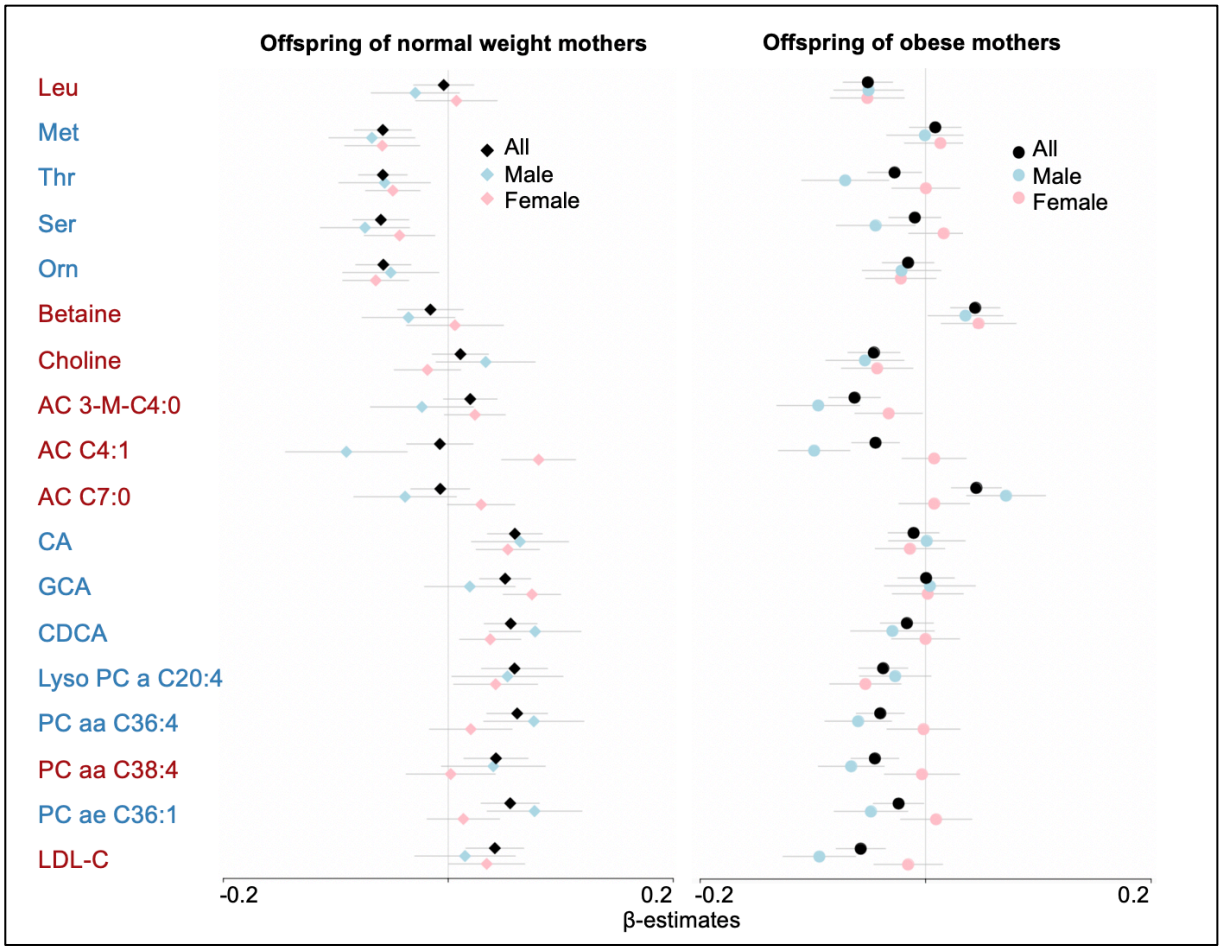

Figure 30: Associations of UCB metabolites and the BMI z-score slope in offspring of age one to four years according to maternal preconception BMI group. Data are scaled β-estimates (SE) of significant associations (p-values < 0.05) depicted as forest plot. Beta-estimates (SE) are based on linear regression models with BMI z-score slope as outcome and metabolite as explanatory variable adjusted for GWG, smoking anytime during pregnancy, breastfeeding (predominantly ≥1 month), and SES performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the regression models. Analysis was repeated after dataset was stratified by offspring sex. Concentrations of LDL-C is provided in mg/dL, bile acids in ng/mL, all other metabolites in µmol/L. Red font indicates metabolite concentrations associated with the BMI z-score slope in offspring of obese, blue font indicates metabolite concentrations associated with the BMI z-score slope in offspring of normal weight mothers. A full list of metabolite abbreviations is provided in Table S 1. BMI, body mass index; GWG, gestational weight gain; SE, standard error; LDL-C, low-density lipoprotein cholesterol; SES, socio-economic status; UCB, umbilical cord blood.

In contrast, BMI development between one and four years of age in offspring of normal weight mothers was inversely associated with concentrations of the amino acids Met, Ser, Thr, Orn

and positively associated with concentrations of the primary bile acids chenodeoxycholic acid (CDCA), cholic acid (CA), and its conjugated form glycocholic acid (GCA), and long-chain fatty acid containing phospholipid species LysoPC a C20:4, PC aa C36:4, and PC ae C36:1. Analysis after stratification for sex revealed that most associations in offspring of obese mothers were only found in male offspring, whereas associations in offspring of normal weight mothers were found in females or unrelated to offspring sex (Table S 11). As AC 3-M-C4:0 showed the strongest effect in offspring of obese mothers, we additionally depicted the weight development of offspring of obese and those of normal weight mothers according to AC 3-M-C4:0 tertiles (Figure 31).

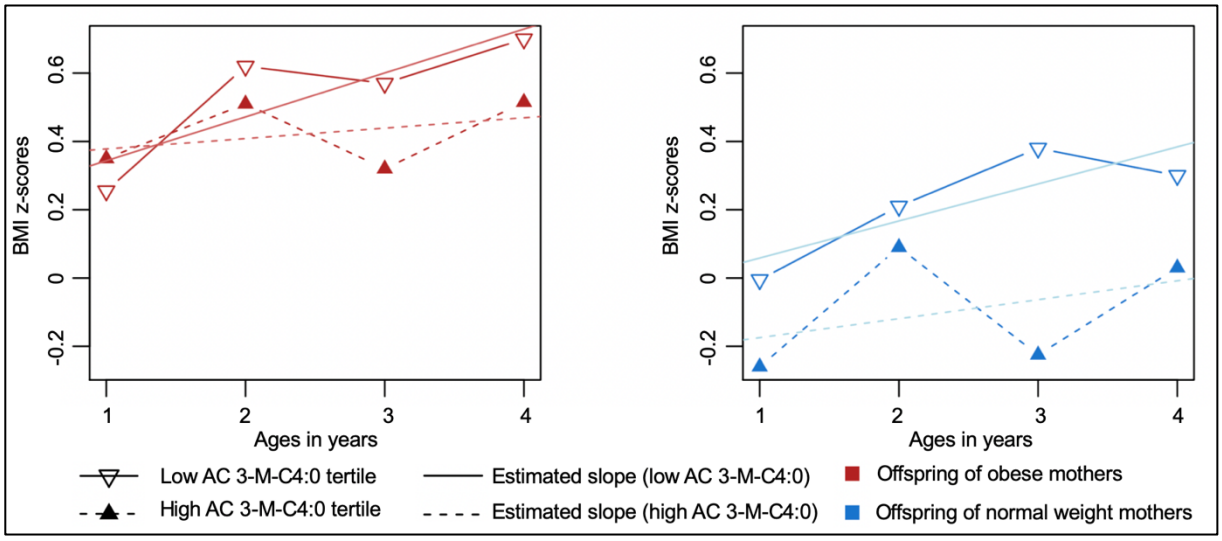

Figure 31: Mean BMI z-scores in offspring of age one to four years according to AC 3-M-C4:0 concentrations at birth. AC 3-M-4:0 (µmol/L) tertiles were calculated based on concentrations of all offspring (n = 398). Mean BMI z-score based on offspring of obese (n = 287) and those of normal weight mothers (n = 111) in the highest and lowest tertile of AC 3-M-C4:0 concentrations at birth were depicted, connected via solid and dotted line, respectively. Estimated slopes of BMI z-scores of offspring of obese and normal weight mothers in the highest and lowest tertile of AC 3-M-C4:0 concentrations at birth, were depicted as solid and dotted line, respectively. BMI, body mass index; AC C3-M-C4:0, isovalerylcarnitine.

We calculated AC 3-M-C4:0 tertiles based on the entire cohort and plotted the mean BMI z-scores at one, two, three, and four years of offspring of the first (lowest) and the ones of the third (highest) tertile in offspring of obese and offspring of normal weight mothers along with the estimated slopes. From two to four years, offspring of obese mothers that had lower AC 3-M-C4:0 concentrations at birth had higher mean BMI z-scores, as shown by a steeper estimated slope. The same pattern was seen in offspring of normal weight mothers, though they had lower mean BMI z-scores throughout.

Betaine and AC 3-M-C4:0 were the two UCB metabolites that were associated with severe maternal preconception obesity (Table 16) and also adverse offspring weight development in offspring of preconception obese mothers (Table S 9, Figure 30). Higher concentrations of

both betaine and AC 3-M-C4:0 were associated with severe maternal preconception obesity, whereas a higher BMI z-score slope from age one to four years in offspring of obese mothers was associated with higher concentrations of UCB betaine but lower concentrations of UCB AC 3-M-C4:0.

3.10.3 Effect of metabolites in UCB on the offspring BMI z-scores at different preschool ages

We next analyzed the relationship between BMI z-scores at ages one, two, three, and four years, and UCB metabolites in offspring of obese and those of normal weight mothers (Table S 12). We performed sensitivity analysis analogously to the study of weight development, as none of the associations remained statistically significant after adjustment for multiple testing. Similarily, most findings remained stable throughout the sensitivity analysis (Table S 13). The associations of metabolite concentrations with BMI z-scores at ages one, two, three, and four years (p-values < 0.05) of offspring of obese and those of normal weight mothers were depicted as forest plots in all, male and female offspring of obese and normal weight mothers (Figure 32). Corresponding β-estimates are provided in the appendix (Table S 14).

The metabolites associated with the BMI z-scores at ages one, two, three, and four years in offspring of obese mothers differed from the ones in offspring of normal weight mothers. Overall, associations were mainly found between lower metabolite concentrations and higher BMI z-scores irrespective of maternal preconception BMI group.

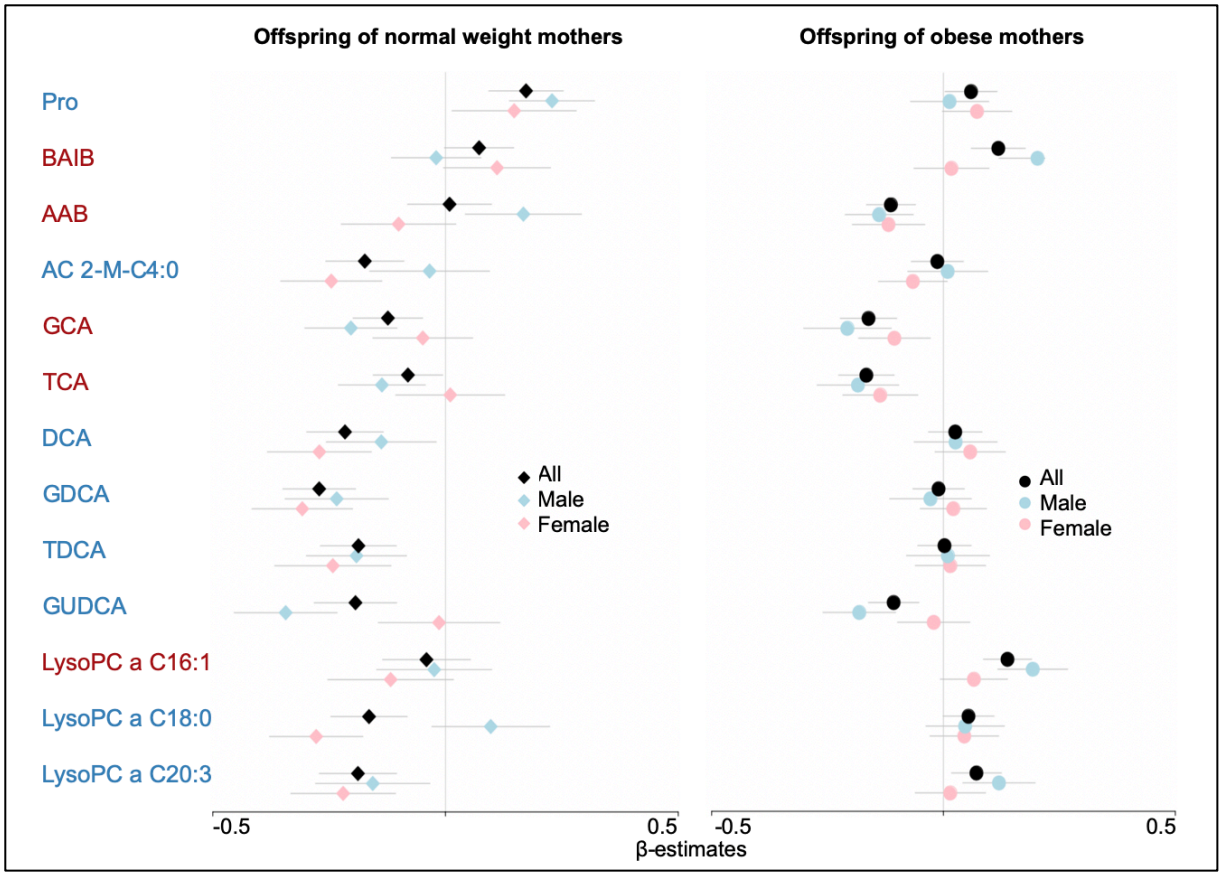

Figure 32: Associations of UCB metabolites and BMI z-scores at one, two, three and four years of age according to maternal preconception BMI group. Data are scaled β-estimates (SE) of significant associations (p-values < 0.05) depicted as forest plot based on linear mixed effect models with BMI z-scores at one, two, three, and four years as outcome and metabolite as explanatory variable adjusted for GWG, smoking anytime during pregnancy, breastfeeding (predominantly ≥1 month), and SES performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the mixed effect models. Analysis was repeated after dataset was stratified by offspring sex. Concentrations of bile acids are provided in ng/mL, all other metabolites in µmol/L. Red font indicates metabolite concentrations associated with BMI z-scores at ages one, two, three and four years in offspring of obese, blue font indicates metabolite concentrations associated with BMI z-scores at ages one, two, three and four years in offspring of normal weight mothers. A full list of metabolite abbreviations is provided in Table S 1. BMI; body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status; UCB, umbilical cord blood.

In offspring of obese mothers, higher BMI z-scores at ages one, two, three, and four years were associated with lower UCB metabolites of α-aminobutyrate (ABB) and the conjugated primary bile acids glycocholic acid (GCA) and taurocholic acid (TCA) and higher concentrations of β-aminoisobutyrate (BAIB) and LysoPC a C16:1. In offspring of normal weight mothers higher BMI z-scores were associated with low concentrations of the acyl-carnitine AC 2-M-C4:0, the secondary bile acids deoxycholic acid (DCA), its conjugates glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA) and the secondary conjugated bile acid GUDCA as well as the long-chain fatty acid containing phospholipid species LysoPC a 18:0 and LysoPC a 20:3. Further, higher UCB concentrations of the amino acid Pro were associated with higher BMI z-scores of offspring of normal weight mothers.

Analysis after stratification for sex showed that in offspring of obese mothers most associations were found in male offspring. In offspring of normal weight mothers associations were found specific for male or female offspring or irrespective of offspring sex (Table S 14). Further, sex-specific rather than maternal BMI-related inverse associations between bile acids and the BMI z-scores were revealed when analyzing male offspring only in both normal weight and obese women. In addition to the association between GCA and the BMI z-scores at the ages one, two, three and four years of age in all and in male offspring of obese mothers, an inverse associations in male offspring of normal weight mothers became apparent, while no association was found when analyzing all offspring of normal weight mothers (Figure 32, Table S 14). Vice versa, in addition to the association between GUDCA and the BMI z-scores at the ages one, two, three and four years of age in all and in male offspring of normal weight mothers, an inverse association in male offspring of obese mothers became apparent while no association was found when analyzing all offspring of obese mothers.

We further analyzed GUDCA due to its stable and strong effect throughout sensitivity analyses (Table S 13) and its sex-specificity (Table S 14). Additional sensitivity analyses of GUDCA in all offspring revealed that the association of GUDCA UCB concentrations at birth and higher offspring BMI z-scores at ages one, two, three, and four years of age was not only independent of maternal preconception BMI but also of intrapartum antibiotics and maternal HbA1c (%) at delivery. However, this finding was only seen in offspring who were breastfed (Figure 33, Table S 15).

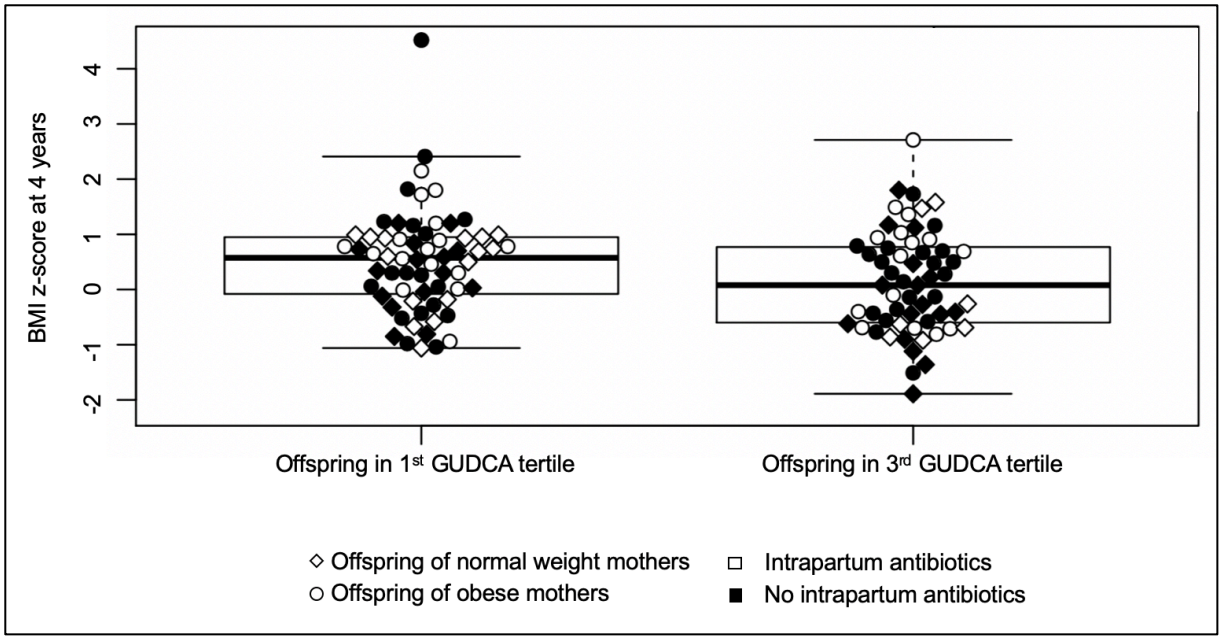

Figure 33: BMI z-scores depending on GUDCA tertiles in breastfed children. GUDCA tertiles were calculated based on UCB concentration (ng/mL) of all offspring (n = 398). Boxplots present the median BMI z-scores of offspring at age four years that were breastfed (n = 184) in the lowest and highest tertile of breastfed children and the distribution of maternal preconception BMI group and intrapartum antibiotic treatment within these groups. Offspring with missing data on intrapartum antibiotic treatment were

excluded from analysis. Data are presented as median (horizontal lines within the boxes), 25th and 75th centile (lower and upper boundaries of the boxes), 1.5 times the interquartile range (whisker ends), and outliers. BMI, body mass index. GUDCA, glycoursodeoxycholic acid.

Further, weight development according to GUDCA tertiles in offspring of obese and offspring of normal weight mothers were depicted (Figure 34).

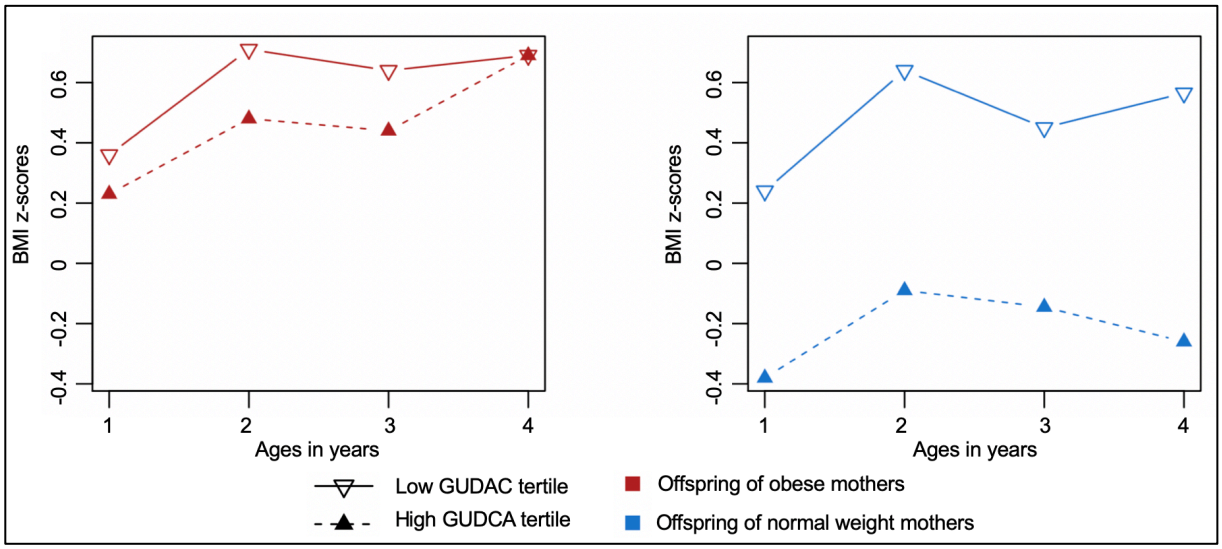

Figure 34: BMI z-scores at the ages one to four according to GUDCA concentrations at birth. GUDCA (ng/mL) tertiles were calculated based on concentration of all offspring (n = 398). BMI z-scores of offspring of obese (n = 287) and those of normal weight mothers (n = 111) in the highest and lowest tertile of GUDCA concentration at birth were depicted. BMI, body mass index. GUDCA, glycoursodeoxycholic acid.

In all offspring, those with lower GUDCA concentrations at birth had higher mean BMI z-scores throughout. BMI z-scores of offspring of obese in the low tertile were slightly higher than in offspring of normal weight mothers, while the difference between the mean BMI z-score in the low and high tertile group were clearly more pronounced in offspring of normal weight mothers.

None of the metabolites associated with the BMI z-scores at one, two, three, and four years of age were associated with (severe) maternal preconception obesity (Table S 5). Further, metabolites associated with higher offspring BMI z-scores at ages one, two, three, and four years differed from the ones associated with the BMI z-score slope from age one to four years. However, both analyses related to weight outcome were related to bile acid concentrations in UCB, a group that was not associated with (severe) maternal preconception obesity at all.

4 Discussion

An obesogenic environment in utero is hypothesized to program fetal metabolism, which predisposes the offspring of mothers with preconception obesity to develop overweight or cardiovascular disease later in life (22, 24). UCB is considered to reflect this intrauterine environment (99) and could thus serve as a biosample to discover surrogate markers for "metabolic programming" of adverse offspring outcomes following adipogenic exposure in utero. The present thesis provides evidence for differences in metabolite concentrations between UCB and adult blood relating to elevated concentrations of amino acids and differences in acyl-carnitine concentrations with noticeably lower concentrations in medium-chain acyl-carnitines. Further, a higher, sex-independent variability of metabolite concentrations was evident in offspring UCB vs. adult blood, both from normal weight, healthy women. Our main analysis identified global deviations in the UCB metabolite profile and alterations in specific metabolites that were related to distinct metabolic pathways as a consequence of the state of obesity of mothers. An adaption of the fetal metabolism was verified by downregulated mRNA expression levels of selected genes of the corresponding pathways and a global hypermethylation of DNA in UCB samples of obese mothers. Maternal dysglycemia during pregnancy of obese women presented an additional factor that seemed to influence the offspring UCB metabolite profile. Maternal preconception BMI- and offspring sex-dependent differences in the relationship between UCB metabolites and offspring preschool BMI development suggest adaptions of the fetal metabolism to an obesogenic intra-uterine environment with potential long-term consequences.

4.1 Differences of metabolites in UCB and adult blood

As a basis for this thesis, concentrations of amino acids and acyl-carnitines were compared between UCB and adult blood. Significant differences were identified between the vast majority of the analyzed amino acid and acyl-carnitine concentrations in UCB samples obtained from offspring of normal weight, healthy mothers and blood of adult women. Particularly, concentrations, related sums and ratios of amino acids were considerably higher in UCB samples compared to female adult blood.

This results extends findings of a study form the 1980's which reported higher concentrations of a smaller set of amino acids in UCB plasma compared to adult plasma (197). Higher concentrations may reflect the consequence of a constant delivery of amino acids to the growing fetus for optimal protein synthesis and oxidation via the placenta (104). Lower concentrations found in Arg, Cit, Gln and the calculated product-to-substrate ratio of ornithine carbamoylphosphate in UCB samples compared to adult blood might be related to a smaller

quantity of amino acids being oxidized and leaving less NH_3 for detoxification in the urea cycle (198).

Most distinct differences were found amongst the acyl-carnitines with lower concentrations of medium-chain acyl-carnitines and higher concentrations of short- and long-chain acyl-carnitines when UCB and adult blood samples were compared. The acyl-carnitine transport via the placenta remains largely unknown though long-chain acyl-carnitines, in contrast to their components i.e. free fatty acid (199) and carnitine, are supposedly not transferred (142). Though all enzymes for β-oxidation of fatty acids are present and active in the human fetus (200, 201), glucose is considered as the prime energy source (104). This would suggest lower β-oxidation product concentrations as compared to those in adult blood as observed for medium-chain acyl-carnitines. Whether β-oxidation, especially of long-chain acyl-carnitines, might play a greater role in the growing fetus or whether these acyl-carnitines are derived from exogenous sources, such as the placenta itself, which exhibits an especially high activity of enzymes involved in the ß-oxidation of long-chain fatty acids (202), remains to be determined.

Compared to amino acid and acyl-carnitine concentrations in adult blood, those in UCB showed higher variability as evident from hierarchical clustering and bi-clustering. Multiple linear regression models did not give any suggestions for clinical reasons for the higher variability of these metabolite groups between UCB samples. None of the various carefully collected and documented pre- or perinatal potential confounding factors investigated in this context, i.e. offspring sex, maternal HbA1c (%) at delivery, GWG, birth weight, GA, or mode of delivery, showed an impact on amino acids and acyl-carnitine concentrations of our study population of offspring of normal weight, healthy mothers. Though some of these potential confounders have also been used by others (99, 100, 144, 203, 204), one needs to stress the fact that our variables are based on well documented clinical data compared to others who might have used self-reported data due to the lack of respective documentation. Further maternal HbA1c (%) at delivery, which reflects the maternal glucose metabolism in the third trimester, is a potential confounder uniquely used in the PEACHES study for analysis of UCB. Potentially, the clinical variables used might show an impact on other metabolite groups, e.g. lipids in UCB, which we have not studied in this analysis, have been shown to be influenced by maternal smoking (205). Additionally, other undiscovered pre-and perinatal or maternal factors might explain biological variability in amino acid and acyl-carnitine concentrations of UCB samples. In our analysis, most of the variance remained unexplained; i.e. could not be linked to any particular confounding factor. Apart from biological reasons, the higher variability between UCB samples could be due to the lower number of adult samples analyzed. Further, though UCB is fetal blood with fetal blood cells which in rare case may contain up to 6%

maternal cells (101, 206), the UCB metabolome comprises fetal, maternal and placental metabolites with each source potentially influenced by different factors. In addition, in the female adult reference group blood was obtained in fasted state whereas UCB samples could not be controlled for fasted/fed state.

Interestingly, UCB amino acids and acyl-carnitines concentrations in the offspring of normal weight, healthy mothers did not show sex-specific changes as shown for adult humans (207, 208). In opposite, we found sex-specific differences in SMs in male and female offspring of severely obese mothers (Table S 6, Table S 7). This is surprising as placental transporters are known to be regulated by hormones, and the endocrine environment exhibits sex-specific effects (209). The lack of sex-specific differences in most of the metabolites suggest that sex-specific differences in these metabolites might develop or manifest only later on or that the sample size in this case was too small to observe such differences.

It needs to be mentioned that our analysis has limitations such as the measurements of UCB and adult samples were performed separately. However, we extensively corrected for this factor statistically and therefore assume that the observed differences are not simply due to analytical biases.

4.2 Effect of maternal preconception obesity on offspring UCB

The notion of a surrogate for “metabolic programming” prompted the investigation of the UCB metabolite profiles in offspring of preconception obese mothers in comparison to normal weight mothers. Our data not only demonstrate an impact of maternal preconception obesity on their offspring’s UCB metabolite profile, but a “dose”-dependent relationship between the extent of maternal preconception obesity and deviations in the UCB metabolite profiles from normal weight mothers. This finding corresponds to the increasing risk for adverse maternal obesity-related outcomes such as GDM (25) and the risk of childhood obesity with higher class of maternal preconception obesity or BMI (41, 42). Preconception obese women are often considered one group, including all women with a BMI ≥30.0 kg/m^2. However, the WHO BMI classification clearly subdivides the group of obese individuals into different classes, according to their risk for comorbidities (167). Our and findings from others further highlight that preconception obese women are not a homogenous group and severity of maternal obesity should be considered in the context of maternal-obesity related changes and adverse outcomes particularly for offspring risks (41, 42).

Another factor worth considering when investigating the metabolic profile is offspring sex since metabolite concentrations are known to exhibit major sexual dimorphisms (207, 208). Though

no sex-specific metabolic profiles have been reported in UCB in relation to maternal preconception obesity, it is evident from our findings that alterations after exposure to an adipogenic environment in utero differ between male and female offspring. The effect of maternal preconception obesity and severe obesity was most pronounced in female offspring, in particular evident from altered concentrations of SMs, a phospholipid species which has been discussed in relation to obesity (210). These findings indicate early sex-specific differences in the metabolism which possibly reflect on differences in later outcome.

Our analysis of individual metabolites revealed increased concentrations of Val and BCAA-derived products and decreased concentrations of one-carbon metabolites in UCB of offspring of severely obese mothers. As we could not differentiate between the monounsaturated C5 acyl-carnitines tiglylcarnitine (AC 2-M-C4:1) and 3-methylcrotonylcarnitine (AC 3-M-C4:1) and the hydroxylated C5 acyl-carnitines 3-hydroxyisovalerylcarnitine (AC 3-M-C4-OH) and 2-hydroxy-2-methylbutyrylcarnitine (AC 2-M-C4-OH), metabolites were taken as AC C5:1 and AC C5-OH and discussed in the context of overall BCAA metabolism rather than specific for Leu and Ile degradation pathways. Elevated concentrations of BCAAs and the derived catabolic products in blood are well established markers for obesity and insulin resistance in adults and children (211). As elevated BCAA concentrations in obese subjects are still apparent after overnight fasting and based on numerous biochemical studies, these changes derive from alterations in the BCAA catabolism rather than higher intake of BCAA (212). Accordingly, downregulated mRNA expression levels of subunits of the BCKDH enzyme, the rate-limiting step of the BCAA catabolism, has been shown in subcutaneous adipose tissue in monozygotic twins discordant for obesity (213) and obese Pima Indians as well as in obese *ob/ob* mice, diabetic *fa/fa* rats and diet-induced obese mice (214). This downregulation was observed along with lower concentration of α-ketoisocaproate, a metabolite derived from Leu in plasma (213). Increased BCAA and decreased metabolite levels derived from the BCAA catabolism in blood reflect tissue-specific changes (215). Elevated BCAA levels might not only be a biomarker of obesity but are discussed as causal agents in the development of obesity, insulin resistance and diabetes by downregulating the AKT signaling pathway, hyperactivation of mechanistic target of rapamycin (mTOR) signaling, inducing oxidative stress, mitochondrial dysfunction, and apoptosis (212).

Recently, elevated concentrations of BCAAs and their degradation products in UCB samples have also been associated with maternal BMI in mid-pregnancy and preconception (99, 144). Our findings of elevated concentrations of the BCAA in UCB may reflect an oversupply from maternal sources, as these essential amino acids can only be derived from maternal circulation, transferred likely via system L amino transporters in the microvillous and basal

plasma membranes of the placenta (103, 104, 106). Whether the concentration of BCAA derived products in UCB stem from maternal or placental metabolism which are transferred to the fetal circulation or whether they are products of fetal BCAA breakdown or a mixture of these routes is unclear, as all entities may catabolize BCAAs (109, 212, 216). Transplacental transfer of BCAA and their degradation products such as AC C0 and AC C2:0 are not understood, yet perfusion experiments suggest that the transfer of small chain acyl-carnitines, such as the BCAA-products, is likely (142).

In contrast to the UCB metabolite profiles, which comprise fetal, maternal and placental metabolites, transcript levels of selected genes from UCB samples reflect fetal mRNA expression only. Thus, our finding of downregulated transcripts of genes involved in the BCAA catabolism in response to maternal obesity as early as in utero complements findings in humans and animals where the origin of BCAA changes in blood are mainly the product of changes in adipose tissue (213, 214). Our findings of elevated levels of Val and related BCAA-products and downregulated mRNA expression of genes involved in the catabolism thus suggest that the alteration in BCAA catabolic mechanisms is an early fetal reactive/adaptive response to cope with the consequences of maternal severe obesity and an oversupply of BCAA which might predispose the offspring for future adverse metabolic outcomes.

Alterations in the concentrations of the one-carbon metabolism-related metabolites in blood samples of obese children and adults including decreased concentrations of Gly, His, Met, and Ser and some phosphatidylcholines connected to one-carbon metabolism have been reported (211). While decreased plasma concentrations of Gly in obese individuals have been well established and attributed to a decreased bioavailability possibly linked to a dysbiosis of the gut microbiota and impaired BCAA metabolism (217), alterations in His, Met, Ser and phospholipid levels are less consistently found. Interpretation of the phospholipid changes is aggravated by the fact that the kit used for targeted LC-MS/MS analysis only provides the sum of the two fatty acid chains by mass but there is no information of the actual fatty acid chains that make up this sum.

In contrast to the BCAAs and derived metabolites, no changes in concentrations of Gly, His, Met, Ser, and phospholipids have been reported in human UCB samples in the context of maternal BMI/obesity though trends for negative associations with phospholipids were provided (144). However, UCB concentrations of Gly, Met, and Ser were decreased in baboon offspring of dams fed a high fat, high energy diet prior to and during pregnancy (218). As maternal concentrations were unchanged, lower concentrations in fetuses were linked to placental dysfunction as reported in relation to transplacental transport activity for neutral,

small amino acids (219) and/or placental inefficiency (220) as reported in human obese pregnancies. Further, in the ovine fetus, an inhibited transplacental transfer of Met has been proposed due to higher maternal BCAA concentrations (221), which might link alterations in the BCAA and one-carbon metabolism in offspring exposed to maternal preconception obesity. A downregulated *DNMT3A* expression level in offspring of severely obese compared to normal weight mothers confirmed our findings of lower Met concentrations in these offspring, as *DNMT3A* expression is regulated by methionine availability (222).

The gene *DNMT3A* encodes for a methyltransferase that catalyzes the transfer of a methyl-groups from SAM to the fifth carbon of cytosine during DNA methylation; an epigenetic modification, susceptible to environmental changes (223, 224). In eukaryotic cells, DNA methylation mainly occurs in CpG isles. *DNMT3A* is primarily known for its activity in *de novo* methylation, which is essential during embryonic development. Activity can be regulated by molecular interactions e.g. with other DNMTs, alternative splicing resulting in isoforms with different enzymatic activity, gene loss and duplication and miRNAs (223, 224). Mutations in *DNMT3A*, characteristic for the Tatton-Brown-Rahman syndrome, result in patient overgrowth and obesity (225). Furthermore, decreased DNMT3A expression in the paraventricular nucleus of the hypothalamus was reported in response to a high fat diet in mice (226). Deletion of the gene resulted in obesity, higher abdominal and subcutaneous fat, hyperphagia, decreased energy expenditure, glucose intolerance, increased insulin and leptin levels suggesting also an important role of the ubiquitously expressed *DNMT3A* in energy homeostasis and control of body weight (226). Our findings of lower concentrations of one-carbon metabolites in combination with downregulated *DNMT3A* expression indicates an impaired *de novo* methylation in response to offspring exposure to maternal severe obesity in utero.

In line with our results of downregulated mRNA expression levels in genes that show different expression levels between offspring of severely obese and normal weight mothers was the finding of a global DNA hypermethylation indicative of a suppression of gene expression. Maternal preconception obesity has so far not been associated with a global change in DNA methylation in UCB although investigated by two studies (227, 228). However, differential methylation of specific CpG sites has been related to maternal preconception obesity where methylation was higher in the majority of differentially methylated genes (90, 91). These hypermethylated sites tended to be positively associated with offspring adiposity during childhood and adolescence while hypomethylated sites were inversely associated (90). Furthermore, UCB based DNA methylation in relation to maternal preconception obesity was found to be sex-specific and associated with adverse effects in childhood BMI z-score and blood pressure in female offspring (91). Noteworthy in this context is also a comparison of

methylation in UCB and peripheral blood in early childhood (229). This longitudinal analysis showed that in the same individual, UCB samples were overall less methylated than peripheral blood at three years, and that methylation at these two timepoints was correlated, which suggests a persistent influence of an adverse intrauterine milieu on offspring outcome (229). As lower methylation might be crucial for adequate gene expression in early, developmental phases, hypermethylated DNA in UCB in offspring of severely obese mothers suggests a disturbed gene expression in response to altered epigenetic modifications due to changes in the intrauterine environment with possible long-term consequences.

Our findings of an altered UCB metabolite profile reflect changes in the intrauterine environment in severely obese pregnancies, linked to maternal overnutrition and associated metabolic complications, impaired placental function, and fetal metabolism. Along with adaptive fetal responses i.e. lower mRNA expression levels of selected genes and higher DNA methylation, our data suggest that the metabolite profile in UCB might function as a surrogate for altered metabolic programming, which predisposes the offspring of severely obese mothers for adverse health outcomes, such as childhood overweight.

4.2.1 Additional effect of late pregnancy hyperglycemia in obese women

As our preliminary work showed that dysglycemia in the last trimester of obese pregnancies despite a negative GDM diagnosis (153, 154) was linked to adverse weight outcome in the offspring (155). We thus assessed the additional effect of maternal dysglycemia on the UCB metabolite profile of offspring exposed to an adipogenic milieu in utero. Offspring of obese mothers with high maternal HbA1c (%) at delivery in offspring of obese mothers was related to of higher concentrations of saturated and monounsaturated medium- and long-chain acyl-carnitines and the product-to-substrate ratio related to the activity of the key enzyme of the carnitine shuttle, CPT1.

Maternal HbA1c (%) at delivery might reflect fetal exposure to glycemic conditions better than maternal GDM diagnosis. By a positive GDM positive diagnosis, treatments to normalize dysglycemia are usually implemented, while in women with a negative GDM test result, maternal dysglycemia may affect the fetus (153-155). The greater influence than by obesity alone on the UCB metabolite profiles was revealed by violin plots. Elevated concentrations of short, medium- and long-chain acyl-carnitines that may result from an impaired β-oxidation are known to change in insulin resistant states (230). As obesity and insulin resistance are closely interlinked it is more difficult to dissect the effects but insulin resistance could regardless of BMI as well change the readout (230). Fetal free fatty acids, which are esterified to carnitines for mitochondrial import to β-oxidation can originate from direct transfer from maternal circulation, from release from placental lipid storages or from fetal hepatic synthesis (231). The

latter has been proposed to occur in late pregnancy due to differences in fetal and maternal fatty acid compositions. In pregnancies of obese mothers, especially under dysglycemic conditions, an excess of glucose crosses the placenta along its concentration gradient inducing fetal hyperinsulinemia which in turn promotes hepatic fatty acid synthesis. However, the lipid hydrolyzing endothelial lipase might also play an important role in the excess of fatty acids in fetal circulation. The endothelial lipase is essential for fatty acid transplacental transfer, as only free fatty acids can be transferred from maternal to fetal circulation with the vast majority of lipids esterified in maternal circulation. Interestingly, lipase activity is only upregulated when maternal obesity is accompanied by GDM (231), supporting the idea of obesity and GDM being independent but additive risk factors for adverse childhood outcomes (99). Though not been reported for UCB samples in this particular setting, medium- and long-chain acyl-carnitines are likely to arise from fetal synthesis (142) as a response to maternal overnutrition. A downregulated mRNA expression level of *CPT2*, the gene which encodes the transferase of the inner mitochondrial membrane that converses acyl-carnitines to acyl-CoA derivatives - and vice versa – suggests an impaired import capacity. The higher product-to-substrate ratio related to the activity of CPT1, the enzyme which catalyzes the conversion of acyl-CoA to the corresponding acyl-carnitines in the outer mitochondrial membrane further supports the concept of a dysregulation in fetal fatty acid catabolism.

Our findings of elevated levels of medium- and long-chain acyl-carnitines and altered expression and activity of involved enzymes indicate that maternal dysglycemia in obese pregnancies has additional negative effects on fetal metabolism. Impaired β-oxidation of fatty acids, possibly due to fetal hyperinsulinemia or an enhanced supply of free fatty acids from maternal circulation may drive fetal metabolism to adapt to this additional adverse environment.

4.3 UCB metabolites and preschool weight outcome

Intrauterine programming is discussed as one of the underlying mechanisms linking maternal obesity and increased offspring risk for obesity starting in early childhood (41, 42). As the UCB metabolite profile may reflect the intrauterine environment, it could serve to identify high-risk offspring as early as at birth. In support of such an approach, we assessed the relationship between UCB metabolite concentrations and a) offspring weight development as measured by the BMI z-score slope between one and four years of age and b) preschool BMI z-scores at ages one, two, three, and four years in offspring of obese mothers as compared to in offspring of normal weight mothers as reference.

As hypothesized from higher obesity risk in offspring exposed to an adipogenic environment in utero (41, 42), we found an expectably higher weight gain during preschool age among

offspring of obese mothers (BMI ≥ 29.5 kg/m^2). This is in accordance with limited findings in the literature, which described a greater growth in BMI in offspring of overweight mothers compared to offspring of normal weight mothers (232).

Following adipogenic exposure, our findings showed different associations between UCB metabolites and weight outcome than in children of normal weight mothers. Furthermore, metabolites related to the BMI z-score slope from age one to four differed from those related to the BMI z-scores of ages one, two, three, and four years. Such differences seem plausible as children might experience high weight gain, i.e. higher BMI z-score slope, while others might have high weight throughout the ages one, two, three, and four years resulting in different metabolite patterns related with adverse weight outcome. Though the association showed no statistically differences after multiple testing, we conducted several sensitivity analyses throughout and found that results remained stable suggesting that our findings were not simply due to chance.

With regard to the BMI z-score slope from age one to four years offspring of obese mothers showed mainly inverse association with metabolites of all four metabolite groups. Offspring of normal weight mothers showed inverse associations with amino acids, while bile acids and phospholipids were positively associated. These patterns were especially interesting with regard to the group of UCB phospholipids where an inversion of the direction of β-estimates associated with the BMI z-score slope from age one to four years was observed when comparing associations of offspring of obese mothers to those of offspring of normal weight mothers. UCB phospholipids were positively associated within offspring of normal weight mothers, whereas in offspring of obese mothers, the association was negative. This finding contributes to the limited understanding from current literature that the direction of association between offspring weight development and UCB metabolites might be inverse after exposure to an adipogenic in utero as proposed in the case of phospholipids (100, 147).

With regard to the BMI z-scores at ages one, two, three, and four years, offspring of normal weight mothers showed mainly inverse associations between UCB metabolites, including bile acids and phospholipids. In offspring of obese mothers, UCB metabolites associated with the BMI z-scores at ages one, two, three, and four years included negative associations with bile acids and positive association with phospholipids. This further supports the opposite role of phospholipid concentrations regarding offspring weight outcome in offspring of obese vs. those of normal weight mothers. Further, the metabolite pattern in children of obese mothers was characterized by a lower variability of metabolite concentrations between female and male offspring compared to female and male offspring of normal weight mothers. Maternal obesity

has been shown to differentially program the offspring's metabolism in male and female offspring resulting in different manifestation of and susceptibilities for metabolic diseases later on (233). The loss or lower manifestation of sex-specific effects has not been described as such. However, in the context of GDM complicated pregnancies, the loss of a sex-specific effect has been reported for the estrogen receptor (ER) alpha expression in maternal decidual endothelial cells (234). Authors suggest altered estrogen levels to be causal, as its expression has been linked to estrogen levels and lower estradiol levels have been described in women with GDM and in UCB of their offspring (234, 235). Interestingly, systemic estrogen deficiency has been discussed in the development of metabolic diseases such as metabolic syndrome and T2D and was described as an obesity triggering factor in postmenopausal stage (236). Thus, a loss of sex-specificity in offspring, in response to maternal preconception obesity, might possibly be linked to disturbed estrogen levels. However, disturbance of mechanisms contributing to sex-specific development of disease such as epigenetic modification, placenta and metabolic hormones (233), might equally be causal for this loss and need to be further investigated.

The metabolite pattern related to an adverse weight outcome was characterized by inverse associations between the weight outcome and metabolite concentrations, especially in offspring of obese mothers. I.e. overall, lower metabolite concentrations were associated with higher offspring BMI z-score slope and BMI z-scores at ages one, two, three, and four years of age. Such inverse associations were also seen in the amino acids related to adverse weight outcome in offspring of normal weight mothers. Lower UCB metabolite concentrations were also associated with adverse weight development in one other study (151). Using an untargeted approach, authors proposed mainly inverse associations between UCB metabolites, including some related to the one-carbon metabolism, and rapid postnatal weight gain in young infants (based on the top quartile of change in weight for age 0-6 months) which resulted in mid-childhood overweight, including obesity (BMI >85th percentile). However, several variables associated with childhood overweight, such as maternal BMI and breastfeeding, differed between studied cases and controls, therefore proposed associations with rapid postnatal weight gain and childhood overweight might possibly not (entirely) trace back to UCB metabolite concentrations but other childhood overweight-related factors (151).

Lower metabolite concentrations might seem contradicting to the notion of an nutritional oversupply in pregnancies of obese women associated with macrosomia, and later obesity and T2D (237). However, as the fetus largely depends on maternal and/or placental nutrient supply, lower concentrations of specific metabolites in the circulation of obese individuals (211), a disturbed transplacental transfer as in placental dysfunction (219) and inefficiency (220), and

an altered placental metabolism (105), might also contribute to metabolic programming of offspring adverse long-term outcome. The relevance and magnitude of the contribution of this novel finding needs additional investigation.

The inverse relationships between bile acid concentrations and BMI z-scores at ages one, two, three, and four years were especially noticeable. Though the metabolite patterns associated with adverse BMI z-score differed between offspring of obese and normal weight mothers, inverse alterations with different bile acid concentrations were observed in both groups in relation to higher BMI z-scores at ages one, two, three, and four years. To our knowledge, bile acids have not been analyzed in the context of maternal obesity and offspring weight outcome. As bile acids are mostly derived from fetal synthesis (121), lower bile acid concentrations are suggestive of an impaired fetal bile acid metabolism. This might be related to higher weight outcome in preschool age. Lower concentrations of primary bile acids in offspring of obese mothers might be linked to a decreased placental steroid production as reported in pregnancies of obese women (238). Though primary bile acids are synthesized from cholesterol in the fetal liver some might be derived from maternal sources (104). Lower concentrations of secondary bile acids, as we found in offspring of normal weight mothers, have been related to a disturbance in the gut microbiota which converts primary into secondary bile acids in the human organism (121). As suggested by our analyses on the example of the secondary bile acid GUDCA, association of UCB bile acids and BMI z-score might be independent of intrapartum antibiotics and maternal BMI but dependent on offspring sex and breastfeeding status. Interestingly, by supplementation with metformin, a positive effect of GUDCA on obesity-related metabolic diseases such as insulin resistance has recently been shown and was explained by its antagonizing effect on farnesoid x receptor (FXR) signaling (239), attributing an important role of adequate bile acid concentration in weight management. Further, the gut microbiome, which is linked to bile acid metabolism, has been associated with the development of childhood obesity (240). However, as many known and possibly unknown pre-, peri- and postnatal factors might independently or additively influence offspring weight development, we can only assume that an impaired bile acid metabolism might somehow contribute to an adverse weight outcome.

Betaine and AC 3-M-C4:0 were the two metabolites associated with both maternal preconception obesity and adverse weight development, i.e. the BMI z-score slope from age one to four years, in offspring of obese mothers. This might suggest that the alterations found in UCB metabolites related to the BCAA catabolism and one-carbon metabolism might extend their effect onto adverse weight development in offspring of obese mothers. As severe maternal preconception obesity was associated with higher UCB concentrations of betaine and

higher UCB concentrations of betaine were associated with the BMI z-score slope from age one to four years in offspring of obese mothers, betaine might potentially mediate adverse weight development in offspring of obese mothers. Though an anti-obesogenic effect of betaine has been described in adults (241), the role of betaine concentrations in UCB for weight development in preschool years is unknown. In contrast, we showed positive associations between AC 3-M-C4:0, a product of Leu catabolism, and severe maternal preconception obesity, whereas the associations between AC 3-M-C4:0 and the BMI z-score slope from age one to four years of offspring of obese mothers was negative. Based on our current knowledge we can only speculate that the finding of high AC 3-M-C4:0 in UCB in offspring of obese mothers might be linked to the development of adverse weight outcome later than preschool age, or that high AC 3-M-C4:0 concentrations might be associated with other maternal-obesity related outcomes in children. However, this needs further investigations.

Based on our findings, we hypothesize that the placenta has a major role in conditioning an adverse weight development in offspring of obese mothers. It presents the barrier between maternal and fetal circulation and is rate limiting in nutrient transfer (104), and this is not only affected by maternal obesity but also by offspring sex (242). Placental dysfunction and inefficiency in pregnancies of obese mothers might predispose offspring to early adverse weight development potentially similar to maternal undernutrition in which a mismatch between prenatal and postnatal nutrient availability predisposes the offspring to rapid postnatal weight gain and adverse health outcomes (76). As the development of overweight and obesity increases with offspring age (41), children born to those mothers might later on even be more susceptible to other adverse outcomes associated with maternal and/or offspring obesity such as T2D (237). In summary, despite a limited number of metabolites and genes analyzed, our findings suggest an in utero programming of offspring weight development in a maternal BMI- and offspring sex-dependent manner.

4.4 Concluding remarks

PEACHES is a large prospective mother-child cohort unique in its size by the number of obese mothers and their offspring. It was designed to investigate the effect of an intrauterine adipogenic environment on short- and long-term consequences with the option to discover markers of adverse outcomes in mother and child to control early preventive measures. The large sample set and database obtained consists of carefully collected clinical data such as anthropometric and cardiometabolic measurements of mother and child, biomaterials collected at different time points and a large variety of variables including potential confounding factors. For the present thesis, a targeted approach was used to profile metabolites in UCB samples and we analyzed them in relation to an obesogenic environment and related to adverse

offspring weight outcomes. Altered metabolite profiles support the hypothesis that childhood overweight/obesity in offspring of obese mothers might be related to processes summarized as metabolic early life programming.

Little is known about the UCB metabolite profiles in offspring exposed to an adipogenic milieu in utero and its relation to offspring weight outcome. Our findings extend the current knowledge of the impact of an adipogenic milieu on the UCB metabolite profile by showing alterations in the BCAA catabolism in response to maternal preconception obesity. Thus far, the BCAA catabolism was only shown to be altered in one study based on maternal mid-pregnancy BMI (99). We further expand the current knowledge on the effect of maternal preconception obesity on the UCB metabolite profile by providing evidence for alterations in the one-carbon metabolism, a metabolic pathway influencing epigenetic processes.

Also, a “dose-dependent” relationship between these UCB metabolite alterations and severity of maternal preconception obesity was established, as we were able to subdivide the group of offspring of obese into obese and severely obese which has not been done previously.

Further, though phospholipids, amino acids and acyl-carnitines are part of targeted metabolite profiling previously used in this setting, we provided novel insights by analyzing bile acids. Though findings were not validated in another cohort, we were able to validate our findings on additional molecular levels introducing the idea of global UCB hypermethylation. This extends to current findings of studies on UCB DNA methylation after exposure to maternal preconception obesity which was shown in epigenomic approaches for specific genes (90, 91).

With regard to weight development, this thesis provides novel insights into the direct comparison of different measures of preschool weight outcome in offspring of normal weight and those of obese mothers in relation to their UCB metabolite profile. Though these findings were not significant after adjustment for multiple testing, we conducted several sensitivity analyses throughout and results remained stable, which suggests that the findings were not simply due to chance, and provide hypotheses for further investigations. Furthermore, though adjustment for multiple testing is necessary with such large quantities of variables, some procedures such as Bonferroni might be too narrow and valuable information might be missed when completely disregarding findings above this cut-off, which might better be investigated exploratively.

Adjustment for potential confounders is a common problem in UCB metabolite profiling, as each metabolite represents an individual variable, which in turn can be influenced by a variety

of factors. However, adjusting each of more than 200 metabolites for potential confounders would be too complex and prone for bias. Thus, the common approach is to use a rather less complete set of pre- and perinatal potential confounders for all metabolites within a study, which may be valid for most entities but not necessary for all. The PEACHES study offers a broad range of well-defined and carefully collected potential pre-, postnatal and perinatal confounding factors, which seem especially important for analysis in the context of UCB metabolite. Such potential confounding factors include measured maternal preconception BMI, GWG based on measured weight, birth weight, mode of delivery, duration of delivery, intrapartum antibiotics, maternal and offspring CRP, breastfeeding, SES, and maternal HbA1c (%) at delivery, the latter, as such, for the analysis of UCB a unique potential confounding factor in the PEACHES cohort. The HbA1c % at delivery reflects the maternal glucose metabolism in the third trimester and might thus be more adequate than the otherwise used GDM diagnosis, as GDM positive women might have a normal or disturbed glucose metabolism following therapy. Further, women, in particular obese ones, with a negative GDM testing might still develop dysglycemia in late pregnancy (154). Though a large proportion of the variance in metabolite concentrations that influence UCB levels remains unexplained, we adjusted our models with carefully selected potential confounding factors including a potential batch effect, which allowed for the best possible adjustment under current knowledge.

A limitation is the targeted approach we used, which analyzed only four prominent metabolite groups. Though this were about 200 metabolites, this panel still presents only a small fraction of a human metabolome. Thus, a targeted approach might not necessarily reveal the most biologically relevant alterations. There may be many other metabolites that may reflect metabolic alterations in offspring exposed to an obesogenic environment and that could be more suitable as surrogates linking maternal obesity to childhood overweight/obesity. However, amino acids, acyl-carnitines and phospholipids are commonly measured in target approaches. This might bias the biological meaning of differences found, as these groups account for the majority of reported marker metabolites; though they might not actually be the metabolites with the highest biological relevance. This seems especially problematic in the case of phospholipids, which represent the largest group of metabolites in most studies using a targeted approach. Phospholipids, in our case mainly phosphatidylcholine species, are composed of a backbone and two esterified fatty acids. However, the analysis only provides the total number of carbon atoms and double bonds, i.e. as the sum of the two fatty acid chains. Interpretation of changes in phospholipid species are particularly difficult, also as for example for most ether-species no biological information is found.

Yet, our approaches used present a practicable, non-invasive method to detect alterations in metabolite concentrations after exposure to an adipogenic milieu in utero and the relation to offspring outcome.

In summary, we identified alterations in the UCB metabolite profiles suggesting changes in a variety of metabolic pathways after an obesogenic exposure in utero according to the severity of maternal obesity. As alterations were also evident in mRNA levels of selected genes we propose that the metabolic profile in UCB samples might possibly function as a surrogate for metabolic programming. Findings of hypermethylated UCB DNA suggest epigenetic changes to be part of adaptive, sex-specific fetal processes that are potentially associated with long-term health consequences as hypothesized by relationships we found with different preschool weight outcomes. Further research is necessary to identify discrete biomarkers for use in the clinical setting to identify newborns at risk for developing overweight that are useful to develop early obesity-preventive concepts.

4.5 Future perspectives

Based on the results of this PhD project, the following approaches for future research are proposed:

1. Findings should be validated in an independent cohort.
2. As maternal obesity is associated with adverse childhood outcome beyond the development of overweight/obesity, it might be worth investigating whether the UCB metabolite profiles associated with maternal obesity are related to other outcomes such as T2D, cardiovascular changes, allergies etc.
3. Since the prospective cohort PEACHES collects anthropometric data beyond the age of four years, the relationship between longitudinal weight outcome and UCB metabolites could further be analyzed in older age groups considering a variety of postnatal confounding factors including lifestyle and nutrition.
4. Furthermore, PEACHES offers the potential to compare the metabolite profiles in UCB to the metabolite profiles in early infancy and childhood. The longitudinal investigation of metabolic profiles might help to elucidate whether alterations in metabolite profiles are persistent and if and how they contribute to metabolic diseases and their development. In case of early infancy, this might contribute to the understanding of whether the UCB metabolic profile reflects fetal adaptions or is rather influenced by maternal and placental metabolites.
5. For a better understanding of underlying biological mechanisms, an untargeted approach might be useful to identify other entities and altered pathways.
6. Generally, a separate analysis of arterial and venous blood, or a simultaneous analysis of UCB and maternal blood in a smaller cohort might be interesting to provide deeper

insights into whether metabolic changes can be attributed to the fetal or the maternal and/or placental metabolism.

With respect to an increasing number of obese or overweight individuals throughout the life cycle new preventive strategies are urgently needed. The finding of an altered metabolite profile in offspring exposed to an adipogenic milieu in utero suggests that UCB samples might be utilized as a source for valid markers that predict the risk to develop overweight or obesity later in life allowing early intervention.

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Appendix

Table S 1: Metabolite abbreviations, biochemical names and analytical characteristica

Abbreviation	Biochemical name	LOD	LOQ	Interassay CV (%)	NA	Mean (SD) total dataset	Mean (SD) total dataset, log2-transformed	Mean (SD) offspring of normal weight mothers, log2-transformed
Acyl-carnitines								
AC 2-M-C3:0	Isobutyrylcarnitine	6.136E-05	1.841E-04	5.907E+00	0	0.057 (0.023)	-4.238 (0.552)	-4.396 (0.528)
AC 2-M-C3:1	Methacrylylcarnitine	3.527E-05	1.058E-04	4.091E+01	5	0.001 (0.000)	-10.831 (0.739)	-10.915 (0.714)
AC 2-M-C4:0	2-Methylbutyrylcarnitine	1.133E-03	3.398E-03	9.213E+00	0	0.107 (0.053)	-3.355 (0.606)	-3.553 (0.600)
AC 3-M-C4:0	Isovalerylcarnitine	6.775E-04	2.033E-03	7.533E+00	0	0.124 (0.137)	-3.465 (1.051)	-3.738 (1.111)
AC C0	Free carnitine	1.344E-02	4.032E-02	5.354E+00	1	20.741 (4.472)	4.344 (0.289)	4.286 (0.287)
AC C10:0	Decanoylcarnitine	1.064E-04	3.193E-04	5.181E+00	1	0.036 (0.016)	-4.905 (0.578)	-4.895 (0.508)
AC C10:1	Decenoylcarnitine	4.792E-04	1.438E-03	6.519E+00	1	0.034 (0.012)	-4.964 (0.491)	-4.972 (0.434)
AC C11:0	Undecanoylcarnitine	1.701E-04	5.103E-04	3.179E+01	1	0.001 (0.001)	-10.163 (0.649)	-10.119 (0.594)
AC C12:0	Dodecanoylcarnitine	1.633E-04	4.899E-04	5.058E+00	0	0.028 (0.014)	-5.322 (0.626)	-5.266 (0.599)
AC C12:1	Dodecenoylcarnitine	2.635E-04	7.906E-04	5.990E+00	1	0.032 (0.015)	-5.108 (0.622)	-5.082 (0.629)
AC C13:0	Tridecanoylcarnitine	2.678E-04	8.033E-04	2.537E+01	1	0.001 (0.000)	-10.476 (0.628)	-10.377 (0.600)
AC C14:0	Tetradecanoylcarnitine	8.888E-04	2.666E-03	4.798E+00	0	0.057 (0.032)	-4.325 (0.715)	-4.288 (0.689)
AC C14:1	Tetradecenoylcarnitine	1.412E-03	4.235E-03	2.849E+01	0	0.059 (0.034)	-4.260 (0.697)	-4.270 (0.667)
AC C15:0	Pentadecanoylcarnitine	6.811E-05	2.043E-04	2.356E+01	2	0.004 (0.003)	-8.038 (0.747)	-7.982 (0.745)
AC C16:0	Hexadecanoylcarnitine	1.048E-03	3.145E-03	6.728E+00	0	0.409 (0.208)	-1.450 (0.672)	-1.477 (0.648)
AC C16:1	Hexadecenoylcarnitine	7.693E-04	2.308E-03	6.711E+00	0	0.067 (0.040)	-4.115 (0.758)	-4.154 (0.768)
AC C17:0	Heptadecanoylcarnitine	9.520E-05	2.856E-04	2.322E+01	0	0.003 (0.001)	-8.788 (0.685)	-8.712 (0.675)
AC C18:0	Octadecanoylcarnitine	1.767E-04	5.301E-04	6.143E+00	0	0.030 (0.012)	-5.156 (0.539)	-5.170 (0.475)

AC C18:1	Octadecenoylcarnitine	9.371E-04	2.811E-03	4.821E+00	0	0.150 (0.073)	-2.891 (0.652)	-2.966 (0.599)
AC C18:2	Octadecadienoylcarnitine	3.332E-03	9.995E-03	4.795E+00	0	0.116 (0.063)	-3.291 (0.715)	-3.302 (0.678)
AC C2:0	Acetylcarnitine	1.459E-03	4.377E-03	6.311E+00	0	2.389 (0.847)	1.183 (0.444)	1.121 (0.457)
AC C3:0	Propionylcarnitine	4.460E-04	1.338E-03	7.343E+00	1	0.361 (0.150)	-1.573 (0.533)	-1.730 (0.482)
AC C3-DC	Malonylcarnitine	4.702E-04	1.411E-03	6.407E+00	2	0.028 (0.008)	-5.220 (0.367)	-5.239 (0.394)
AC C3-M-DC	Methylmalonylcarnitine	1.821E-03	5.462E-03	2.039E+01	1	0.009 (0.003)	-6.930 (0.413)	-6.884 (0.431)
AC C4:0	Butyrylcarnitine	5.991E-05	1.797E-04	5.101E+00	3	0.056 (0.027)	-4.273 (0.539)	-4.331 (0.575)
AC C4:1	Crotonylcarnitine	8.215E-05	2.465E-04	2.022E+02	3	0.001 (0.001)	-11.324 (1.292)	-11.521 (1.131)
AC C4-DC	Succinylcarnitine	1.659E-03	4.978E-03	5.869E+00	3	0.070 (0.038)	-3.949 (0.536)	-3.965 (0.509)
AC C4-OHa	3-Hydroxybutyrylcarnitine a (L-isomere)	6.655E-05	1.997E-04	7.727E+00	0	0.018 (0.012)	-6.048 (0.797)	-6.108 (0.767)
AC C4-OHb	3-Hydroxybutyrylcarnitine b (D-isomere)	6.467E-05	1.940E-04	1.212E+01	0	0.041 (0.046)	-5.116 (1.164)	-5.299 (1.109)
AC C5:1	Tiglylcarnitine + 3-methylcrotonylcarnitine (Non-distinguishable stereoisomeres)	1.696E-04	5.089E-04	7.673E+00	0	0.016 (0.008)	-6.121 (0.599)	-6.276 (0.521)
AC C5-DC	Glutarylcarnitine	7.647E-04	2.294E-03	5.811E+00	1	0.106 (0.036)	-3.305 (0.455)	-3.366 (0.510)
AC C5-M-DC	Methylglutarylcarnitine	5.213E-04	1.564E-03	4.146E+01	0	0.054 (0.046)	-4.648 (1.126)	-4.156 (1.123)
AC C5-OH	3-Hydroxyisovalerylcarnitine + 3-hydroxy-2-methylbutyrylcarnitine (Non-distinguishable stereoisomeres)	1.704E-04	5.113E-04	7.952E+00	1	0.032 (0.012)	-5.056 (0.495)	-5.201 (0.471)
AC C6:0	Hexanoylcarnitine	1.067E-04	3.201E-04	6.147E+00	0	0.043 (0.027)	-4.738 (0.751)	-4.837 (0.675)
AC C6:1	Hexenoylcarnitine	1.416E-04	4.247E-04	2.145E+01	1	0.011 (0.006)	-6.617 (0.659)	-6.558 (0.672)
AC C6-DC	Adipylcarnitine	4.173E-04	1.252E-03	7.033E+00	0	0.049 (0.042)	-4.713 (0.959)	-4.886 (0.925)
AC C6-OHa	Hydroxyhexanoylcarnitine a (L-isomere)	7.764E-05	2.329E-04	5.230E+00	0	0.005 (0.003)	-7.869 (0.707)	-8.049 (0.586)
AC C6-OHb	Hydroxyhexanoylcarnitine b (D-isomere)	9.600E-05	2.880E-04	6.469E+00	0	0.002 (0.001)	-8.781 (0.586)	-8.894 (0.549)
AC C7:0	Heptanoylcarnitine	1.174E-05	3.523E-05	8.589E+01	1	0.001 (0.000)	-10.827 (0.569)	-10.829 (0.572)
AC C7-DC	Pimeloylcarnitine	2.862E-04	8.586E-04	6.258E+00	0	0.013 (0.006)	-6.335 (0.569)	-6.253 (0.536)
AC C8:0	Octanoylcarnitine	5.239E-05	1.572E-04	1.202E+01	0	0.013 (0.006)	-6.373 (0.609)	-6.387 (0.549)
AC C8:1	Octenoylcarnitine	5.147E-04	1.544E-03	1.612E+01	0	0.025 (0.015)	-5.546 (0.819)	-5.756 (0.787)
AC C8-OH	Hydroxyoctanoylcarnitine	8.931E-05	2.679E-04	6.354E+00	0	0.006 (0.002)	-7.591 (0.534)	-7.587 (0.443)
AC C9:0	Nonanoylcarnitine	6.514E-05	1.954E-04	2.780E+01	1	0.001 (0.001)	-9.787 (0.757)	-9.622 (0.739)

AC iso-C11:0	Isoundecanoylcarnitine	2.262E-04	6.785E-04	1.709E+01	1	0.004 (0.002)	-8.017 (0.679)	-7.955 (0.700)
AC iso-C13:0	Isotridecanoylcarnitine	2.405E-04	7.216E-04	1.575E+01	1	0.010 (0.007)	-6.811 (0.757)	-6.593 (0.664)
AC iso-C15:0	Isopentadecanoylcarnitine	1.293E-04	3.879E-04	1.139E+01	0	0.004 (0.002)	-8.198 (0.682)	-8.070 (0.634)
AC iso-C17:0	15-Methylhexadecanoylcarnitine	9.414E-05	2.824E-04	2.393E+01	2	0.003 (0.001)	-8.559 (0.683)	-8.496 (0.693)
AC iso-C9:0	Isononanoylcarnitine	7.403E-05	2.221E-04	7.698E+00	1	0.005 (0.003)	-7.927 (0.707)	-7.832 (0.754)
Amino acids								
1-M-His	1-Methylhistidine	1.951E-01	5.854E-01	1.330E+01	0	3.777 (1.171)	1.846 (0.462)	1.824 (0.450)
3-M-His	3-Methylhistidine	3.429E-02	1.029E-01	7.344E+00	8	5.074 (7.480)	1.224 (1.852)	0.912 (1.999)
AAB	α-Aminobutyric acid	2.401E-02	7.203E-02	7.956E+00	0	21.205 (8.073)	4.311 (0.524)	4.299 (0.482)
AADP	α-Aminoadipic acid	1.419E-02	4.258E-02	2.510E+01	1	1.843 (0.757)	0.782 (0.529)	0.732 (0.483)
Ala	Alanine	2.611E-01	7.833E-01	9.451E+00	0	638.874 (161.723)	9.274 (0.362)	9.357 (0.371)
Anserine	Anserine	2.199E-02	6.596E-02	2.480E+02	68	0.113 (0.219)	-4.177 (1.693)	-4.201 (1.893)
Arg	Arginine	1.797E+00	5.391E+00	1.506E+01	0	31.449 (16.111)	4.770 (0.809)	4.952 (0.762)
Asn	Asparagine	4.055E-02	1.216E-01	3.042E+01	1	77.999 (22.135)	6.232 (0.390)	6.364 (0.409)
Asp	Aspartate	2.374E-02	7.123E-02	8.991E+00	0	81.313 (25.286)	6.284 (0.415)	6.414 (0.411)
BAIB	β-Aminoisobutyric acid	5.019E-02	1.506E-01	1.752E+01	1	1.283 (1.254)	0.022 (0.905)	0.111 (1.017)
Carnosine	Carnosine	2.481E-03	7.443E-03	2.041E+02	15	0.444 (0.445)	-1.892 (1.626)	-2.011 (1.449)
Cit	Citrulline	3.274E-01	9.821E-01	1.037E+01	0	14.974 (3.680)	3.861 (0.354)	3.891 (0.358)
Cys-Cys	Cysteine-Cysteine	1.059E-02	3.178E-02	2.357E+01	2	18.819 (6.864)	4.158 (0.452)	4.210 (0.506)
Dimethylglycine	Dimethylglycine	1.028E-02	3.084E-02	1.183E+01	3	2.738 (1.016)	1.366 (0.496)	1.320 (0.575)
GABA	γ-Aminobutyric Acid	1.144E-03	3.432E-03	5.587E+01	23	0.445 (0.257)	-1.459 (1.051)	-1.270 (0.993)
Gln	Glutamine	2.630E-01	7.891E-01	1.754E+01	0	340.584 (90.184)	8.361 (0.391)	8.396 (0.423)
Glu	Glutamate	3.340E-01	1.002E+00	7.124E+00	0	369.467 (82.662)	8.491 (0.339)	8.559 (0.303)
Gly	Glycine	4.688E-01	1.407E+00	7.235E+00	0	392.590 (72.662)	8.592 (0.266)	8.704 (0.262)
Hcys	Homocysteine	9.784E-01	2.935E+00	1.391E+01	3	59.410 (47.013)	5.585 (0.913)	5.537 (0.903)
His	Histidine	1.029E-01	3.086E-01	1.799E+01	0	157.988 (34.980)	7.269 (0.318)	7.396 (0.292)
Ile	Isoleucine	3.426E-01	1.028E+00	1.527E+01	0	75.841 (19.538)	6.198 (0.369)	6.196 (0.329)
Leu	Leucine	4.836E-02	1.451E-01	7.084E+00	1	135.500 (30.158)	7.045 (0.333)	7.067 (0.322)
Lys	Lysine	1.595E-01	4.785E-01	1.031E+01	2	398.456 (66.681)	8.618 (0.241)	8.626 (0.241)

Met	Methionine	1.400E-02	4.201E-02	1.064E+01	0	42.856 (8.516)	5.395 (0.276)	5.471 (0.274)
OH-Pro	4-Hydroxyproline	1.297E-02	3.892E-02	1.133E+01	0	25.153 (5.117)	4.624 (0.288)	4.503 (0.255)
Orn	Ornithine	1.440E-01	4.319E-01	6.535E+00	0	192.990 (36.071)	7.568 (0.267)	7.645 (0.267)
Phe	Phenylalanine	3.084E-02	9.251E-02	5.823E+00	0	110.654 (16.972)	6.773 (0.221)	6.816 (0.223)
Pro	Proline	1.908E-01	5.723E-01	6.819E+00	0	191.668 (29.145)	7.566 (0.216)	7.615 (0.225)
Sarcosine	Sarcosine	1.224E-02	3.672E-02	1.083E+01	3	0.790 (0.245)	-0.404 (0.426)	-0.372 (0.390)
Ser	Serine	1.750E-01	5.250E-01	1.971E+01	0	240.807 (66.367)	7.854 (0.422)	7.960 (0.441)
Thr	Threonine	8.702E-02	2.611E-01	7.770E+00	0	322.093 (73.308)	8.295 (0.325)	8.296 (0.366)
Trp	Tryptophan	2.441E-01	7.324E-01	7.149E+00	1	62.430 (11.640)	5.939 (0.273)	5.965 (0.266)
Tyr	Tyrosine	1.951E-02	5.853E-02	8.777E+00	0	85.626 (15.666)	6.396 (0.262)	6.402 (0.228)
Val	Valine	1.257E-01	3.770E-01	6.206E+00	0	241.722 (38.279)	7.899 (0.231)	7.869 (0.235)
β-Ala	β-Alanine	5.104E-02	1.531E-01	1.936E+01	6	0.660 (0.303)	-0.793 (0.853)	-0.730 (0.901)
Amino acids derivatives								
Betaine	Betaine	1.878E-01	5.635E-01	1.116E+01	3	32.320 (8.210)	4.968 (0.368)	4.909 (0.347)
Choline	Choline	9.711E-02	2.913E-01	2.209E+01	4	755.629 (177.084)	9.524 (0.331)	9.524 (0.351)
Bile acids								
CA	Cholic acid	1.691E+00	5.073E+00	7.727E+00	1	30.245 (28.747)	4.549 (0.983)	4.598 (1.042)
CDCA	Chenodeoxycholic acid	8.059E-01	2.418E+00	7.340E+00	2	17.028 (7.790)	3.965 (0.591)	3.836 (0.651)
DCA	Deoxycholic acid	6.683E+00	2.005E+01	8.743E+00	30	20.670 (25.717)	3.603 (1.607)	3.573 (1.619)
GCA	Glycocholic acid	1.415E+00	4.246E+00	6.711E+00	1	277.069 (323.244)	7.667 (1.058)	7.821 (1.236)
GCDCA	Glycochenodeoxycholic acid	1.761E+00	5.283E+00	7.051E+00	0	413.379 (303.207)	8.347 (1.037)	8.271 (1.107)
GDCA	Glycodeoxycholic acid	1.915E+00	5.745E+00	6.680E+00	8	6.576 (8.735)	1.972 (1.486)	2.243 (1.497)
GLCA	Glycolithocholic acid	2.436E-01	7.307E-01	7.739E+00	203	0.410 (0.469)	-1.987 (1.566)	-1.649 (1.358)
GUDCA	Glycoursodeoxycholic acid	4.100E-01	1.230E+00	7.144E+00	8	4.058 (9.210)	1.285 (1.362)	1.271 (1.313)
LCA	Lithocholic acid	1.052E+00	3.157E+00	9.215E+00	169	2.102 (2.861)	0.319 (1.628)	0.202 (1.656)
TCA	Taurocholic acid	3.228E-01	9.684E-01	7.483E+00	0	408.037 (344.095)	8.282 (1.057)	8.368 (1.233)
TCDCA	Taurochenodeoxycholic acid	3.799E-01	1.140E+00	7.302E+00	0	800.994 (594.538)	9.333 (0.947)	9.254 (0.986)
TDCA	Taurodeoxycholic acid	3.687E-01	1.106E+00	7.021E+00	17	4.207 (4.169)	1.380 (1.582)	1.551 (1.614)
TLCA	Taurolithocholic acid	9.274E-02	2.782E-01	1.348E+01	70	1.244 (1.425)	-0.299 (1.407)	-0.187 (1.476)

TLCA-S	Taurolithocholic acid-sulfate	1.473E-01	4.418E-01	7.986E+00	0	52.239 (53.476)	5.326 (0.990)	5.693 (1.000)
TUDCA	Tauroursodeoxycholic acid	3.145E-01	9.436E-01	8.040E+00	17	3.500 (3.251)	1.503 (0.906)	1.669 (0.841)
UDCA	Ursodeoxycholic acid	8.151E-01	2.445E+00	7.418E+00	157	7.665 (7.777)	2.455 (1.161)	2.248 (1.137)
Lipids								
HDL cholesterol	High-density lipoprotein cholesterol	1.000E-01	3.000E-03	<2.5	1	28.690 (8.265)	4.788 (0.394)	4.815 (0.382)
LDL cholesterol	Low-density lipoprotein cholesterol	5.000E-01	1.000E-02	<3	1	36.280 (11.254)	5.116 (0.435)	5.149 (0.424)
TG	Triglyceride	1.000E+00	1.000E-02	<2	2	38.328 (21.709)	5.074 (0.711)	5.204 (0.709)
Phospholipids								
LysoPC a C14:0	Lysophosphatidylcholine (acyl) C14:0	1.180E-01	3.539E-01	7.560E+00	1	2.651 (0.601)	1.372 (0.314)	1.383 (0.277)
LysoPC a C16:0	Lysophosphatidylcholine (acyl) C16:0	2.101E+00	6.302E+00	8.262E+00	0	111.383 (27.523)	6.757 (0.352)	6.735 (0.337)
LysoPC a C16:1	Lysophosphatidylcholine (acyl) C16:1	1.598E-01	4.794E-01	7.776E+00	2	5.878 (1.630)	2.505 (0.378)	2.442 (0.343)
LysoPC a C17:0	Lysophosphatidylcholine (acyl) C17:0	9.244E-02	2.773E-01	7.739E+00	0	1.454 (0.381)	0.496 (0.351)	0.577 (0.358)
LysoPC a C18:0	Lysophosphatidylcholine (acyl) C18:0	5.775E-01	1.732E+00	8.107E+00	0	17.227 (5.027)	4.048 (0.414)	4.042 (0.410)
LysoPC a C18:1	Lysophosphatidylcholine (acyl) C18:1	4.617E-01	1.385E+00	8.359E+00	0	13.268 (3.390)	3.686 (0.354)	3.593 (0.316)
LysoPC a C18:2	Lysophosphatidylcholine (acyl) C18:2	6.429E-01	1.929E+00	8.442E+00	0	9.713 (3.095)	3.207 (0.468)	3.194 (0.456)
LysoPC a C18:3	Lysophosphatidylcholine (acyl) C18:3	6.480E-02	1.944E-01	1.003E+01	1	0.259 (0.084)	-2.022 (0.470)	-2.033 (0.474)
LysoPC a C20:3	Lysophosphatidylcholine (acyl) C20:3	7.357E-02	2.207E-01	9.551E+00	0	2.180 (0.705)	1.053 (0.458)	1.011 (0.461)
LysoPC a C20:4	Lysophosphatidylcholine (acyl) C20:4	1.628E-01	4.885E-01	8.417E+00	1	10.526 (3.232)	3.329 (0.448)	3.217 (0.421)
LysoPC a C20:5	Lysophosphatidylcholine (acyl) C20:5	5.216E-02	1.565E-01	9.786E+00	1	0.279 (0.116)	-1.957 (0.586)	-1.930 (0.641)
LysoPC a C22:5	Lysophosphatidylcholine (acyl) C22:5	5.219E-02	1.566E-01	1.551E+01	0	0.182 (0.071)	-2.567 (0.576)	-2.653 (0.612)
LysoPC a C22:6	Lysophosphatidylcholine (acyl) C22:6	7.860E-02	2.358E-01	1.175E+01	0	1.407 (0.479)	0.413 (0.483)	0.423 (0.488)
LysoPC a C24:0	Lysophosphatidylcholine (acyl) C24:0	5.179E-02	1.554E-01	1.790E+01	0	0.191 (0.034)	-2.413 (0.269)	-2.411 (0.249)
LysoPC a C26:0	Lysophosphatidylcholine (acyl) C26:0	5.046E-02	1.514E-01	2.905E+01	4	0.173 (0.084)	-2.644 (0.535)	-2.687 (0.463)
LysoPC a C26:1	Lysophosphatidylcholine (acyl) C26:1	5.395E-02	1.618E-01	1.098E+01	1	0.540 (0.053)	-0.896 (0.142)	-0.934 (0.130)
LysoPC a C28:0	Lysophosphatidylcholine (acyl) C28:0	6.402E-02	1.921E-01	1.419E+01	0	0.481 (0.076)	-1.074 (0.222)	-1.061 (0.223)
LysoPC a C28:1	Lysophosphatidylcholine (acyl) C28:1	4.952E-02	1.485E-01	1.791E+01	0	0.159 (0.051)	-2.726 (0.450)	-2.733 (0.465)
PC aa C24:0	Phosphatidylcholine (diacyl) C24:0	6.732E-03	2.020E-02	2.759E+01	0	0.040 (0.018)	-4.777 (0.588)	-4.851 (0.640)
PC aa C26:0	Phosphatidylcholine (diacyl) C26:0	9.732E-03	2.920E-02	1.970E+01	3	0.148 (0.035)	-2.788 (0.307)	-2.841 (0.302)
PC aa C28:1	Phosphatidylcholine (diacyl) C28:1	1.322E-02	3.965E-02	8.043E+00	0	0.873 (0.227)	-0.241 (0.354)	-0.178 (0.358)

PC aa C30:0	Phosphatidylcholine (diacyl) C30:0	2.004E-02	6.012E-02	7.376E+00	0	4.200 (1.184)	2.017 (0.386)	2.069 (0.360)
PC aa C32:0	Phosphatidylcholine (diacyl) C32:0	1.224E-01	3.672E-01	8.496E+00	0	40.777 (12.558)	5.285 (0.430)	5.282 (0.410)
PC aa C32:1	Phosphatidylcholine (diacyl) C32:1	1.359E-01	4.076E-01	9.271E+00	0	29.009 (13.448)	4.718 (0.635)	4.719 (0.576)
PC aa C32:3	Phosphatidylcholine (diacyl) C32:3	1.736E-02	5.207E-02	1.119E+01	0	0.471 (0.128)	-1.135 (0.370)	-1.112 (0.355)
PC aa C34:1	Phosphatidylcholine (diacyl) C34:1	1.238E+00	3.715E+00	8.692E+00	0	400.786 (105.527)	8.600 (0.366)	8.595 (0.342)
PC aa C34:2	Phosphatidylcholine (diacyl) C34:2	6.830E+00	2.049E+01	9.664E+00	1	288.063 (93.211)	8.099 (0.454)	8.152 (0.413)
PC aa C34:3	Phosphatidylcholine (diacyl) C34:3	1.298E-01	3.894E-01	8.735E+00	1	8.496 (3.142)	2.999 (0.500)	3.043 (0.426)
PC aa C34:4	Phosphatidylcholine (diacyl) C34:4	2.681E-02	8.043E-02	9.645E+00	1	1.659 (0.474)	0.676 (0.394)	0.678 (0.331)
PC aa C36:0	Phosphatidylcholine (diacyl) C36:0	4.837E-02	1.451E-01	4.015E+01	0	4.600 (1.513)	2.121 (0.494)	2.252 (0.470)
PC aa C36:1	Phosphatidylcholine (diacyl) C36:1	2.781E-01	8.342E-01	1.757E+01	0	73.828 (18.601)	6.163 (0.351)	6.178 (0.328)
PC aa C36:2	Phosphatidylcholine (diacyl) C36:2	1.054E+00	3.161E+00	9.121E+00	1	167.836 (48.801)	7.333 (0.410)	7.390 (0.370)
PC aa C36:3	Phosphatidylcholine (diacyl) C36:3	6.888E-01	2.066E+00	9.143E+00	0	233.336 (66.389)	7.809 (0.407)	7.848 (0.410)
PC aa C36:4	Phosphatidylcholine (diacyl) C36:4	9.154E-01	2.746E+00	9.766E+00	0	631.817 (154.241)	9.260 (0.355)	9.228 (0.329)
PC aa C36:5	Phosphatidylcholine (diacyl) C36:5	1.894E-01	5.683E-01	9.928E+00	0	20.523 (8.267)	4.261 (0.525)	4.376 (0.566)
PC aa C36:6	Phosphatidylcholine (diacyl) C36:6	2.142E-02	6.427E-02	1.072E+01	1	1.062 (0.319)	0.025 (0.420)	0.088 (0.389)
PC aa C38:0	Phosphatidylcholine (diacyl) C38:0	2.497E-02	7.492E-02	9.674E+00	0	7.450 (1.878)	2.854 (0.350)	2.932 (0.342)
PC aa C38:1	Phosphatidylcholine (diacyl) C38:1	3.887E-02	1.166E-01	1.982E+01	159	0.787 (0.544)	-0.830 (1.393)	-1.094 (1.510)
PC aa C38:3	Phosphatidylcholine (diacyl) C38:3	1.798E-01	5.393E-01	9.789E+00	0	140.380 (37.369)	7.084 (0.378)	7.134 (0.370)
PC aa C38:4	Phosphatidylcholine (diacyl) C38:4	3.817E-01	1.145E+00	9.757E+00	0	443.657 (106.665)	8.752 (0.350)	8.726 (0.317)
PC aa C38:5	Phosphatidylcholine (diacyl) C38:5	2.151E-01	6.452E-01	1.052E+01	0	88.541 (21.201)	6.429 (0.337)	6.425 (0.316)
PC aa C38:6	Phosphatidylcholine (diacyl) C38:6	2.578E-01	7.734E-01	1.027E+01	0	290.746 (92.626)	8.115 (0.443)	8.209 (0.430)
PC aa C40:1	Phosphatidylcholine (diacyl) C40:1	1.801E-02	5.403E-02	1.207E+01	0	0.735 (0.175)	-0.482 (0.334)	-0.403 (0.298)
PC aa C40:2	Phosphatidylcholine (diacyl) C40:2	1.700E-02	5.101E-02	1.554E+01	1	0.376 (0.116)	-1.481 (0.463)	-1.489 (0.446)
PC aa C40:3	Phosphatidylcholine (diacyl) C40:3	1.929E-02	5.786E-02	1.074E+01	0	1.609 (0.364)	0.651 (0.317)	0.648 (0.306)
PC aa C40:4	Phosphatidylcholine (diacyl) C40:4	2.621E-02	7.863E-02	1.082E+01	0	10.627 (2.833)	3.362 (0.367)	3.397 (0.330)
PC aa C40:5	Phosphatidylcholine (diacyl) C40:5	3.037E-02	9.110E-02	1.091E+01	0	17.815 (6.106)	4.074 (0.482)	4.081 (0.480)
PC aa C40:6	Phosphatidylcholine (diacyl) C40:6	7.469E-02	2.241E-01	1.135E+01	0	105.489 (37.357)	6.637 (0.491)	6.751 (0.465)
PC aa C42:0	Phosphatidylcholine (diacyl) C42:0	1.530E-02	4.589E-02	1.335E+01	0	1.058 (0.349)	0.012 (0.441)	0.131 (0.396)
PC aa C42:1	Phosphatidylcholine (diacyl) C42:1	1.470E-02	4.410E-02	2.180E+01	0	0.799 (0.238)	-0.383 (0.406)	-0.308 (0.388)

PC aa C42:2	Phosphatidylcholine (diacyl) C42:2	1.485E-02	4.455E-02	1.549E+01	0	0.535 (0.131)	-0.943 (0.345)	-0.934 (0.295)
PC aa C42:4	Phosphatidylcholine (diacyl) C42:4	1.567E-02	4.701E-02	2.219E+01	1	0.956 (0.228)	-0.104 (0.327)	-0.103 (0.297)
PC aa C42:5	Phosphatidylcholine (diacyl) C42:5	1.626E-02	4.878E-02	1.498E+01	0	1.128 (0.314)	0.123 (0.376)	0.122 (0.317)
PC aa C42:6	Phosphatidylcholine (diacyl) C42:6	1.654E-02	4.962E-02	1.577E+01	0	0.885 (0.231)	-0.222 (0.357)	-0.203 (0.318)
PC ae C30:0	Phosphatidylcholine (acyl-alkyl) C30:0	8.605E-03	2.582E-02	8.860E+00	0	0.327 (0.093)	-1.668 (0.389)	-1.581 (0.384)
PC ae C30:1	Phosphatidylcholine (acyl-alkyl) C30:1	1.071E-02	3.214E-02	9.284E+00	338	0.024 (0.023)	-6.247 (2.125)	-7.552 (2.757)
PC ae C30:2	Phosphatidylcholine (acyl-alkyl) C30:2	7.971E-03	2.391E-02	1.344E+01	0	0.065 (0.015)	-3.967 (0.318)	-3.923 (0.300)
PC ae C32:1	Phosphatidylcholine (acyl-alkyl) C32:1	3.015E-02	9.045E-02	8.992E+00	0	5.809 (1.859)	2.470 (0.443)	2.490 (0.433)
PC ae C32:2	Phosphatidylcholine (acyl-alkyl) C32:2	1.160E-02	3.480E-02	1.028E+01	0	1.214 (0.382)	0.213 (0.434)	0.239 (0.418)
PC ae C34:0	Phosphatidylcholine (acyl-alkyl) C34:0	2.370E-02	7.111E-02	1.041E+01	0	2.845 (1.010)	1.428 (0.475)	1.560 (0.457)
PC ae C34:1	Phosphatidylcholine (acyl-alkyl) C34:1	7.770E-02	2.331E-01	8.624E+00	0	14.650 (4.385)	3.813 (0.413)	3.833 (0.382)
PC ae C34:2	Phosphatidylcholine (acyl-alkyl) C34:2	9.401E-02	2.820E-01	9.724E+00	1	9.589 (2.715)	3.207 (0.392)	3.232 (0.362)
PC ae C34:3	Phosphatidylcholine (acyl-alkyl) C34:3	5.088E-02	1.526E-01	9.320E+00	1	3.633 (1.270)	1.782 (0.473)	1.889 (0.435)
PC ae C36:0	Phosphatidylcholine (acyl-alkyl) C36:0	2.507E-02	7.521E-02	1.498E+01	0	2.184 (0.581)	1.079 (0.371)	1.097 (0.349)
PC ae C36:1	Phosphatidylcholine (acyl-alkyl) C36:1	7.250E-02	2.175E-01	9.616E+00	0	8.552 (2.427)	3.042 (0.393)	3.128 (0.375)
PC ae C36:2	Phosphatidylcholine (acyl-alkyl) C36:2	9.541E-02	2.862E-01	9.594E+00	1	8.364 (2.553)	3.001 (0.425)	3.098 (0.385)
PC ae C36:3	Phosphatidylcholine (acyl-alkyl) C36:3	6.846E-02	2.054E-01	1.021E+01	1	5.926 (1.747)	2.507 (0.415)	2.579 (0.388)
PC ae C36:4	Phosphatidylcholine (acyl-alkyl) C36:4	1.367E-01	4.100E-01	1.047E+01	0	39.721 (9.871)	5.269 (0.353)	5.284 (0.321)
PC ae C36:5	Phosphatidylcholine (acyl-alkyl) C36:5	8.640E-02	2.592E-01	1.046E+01	0	26.346 (7.577)	4.662 (0.408)	4.666 (0.407)
PC ae C38:0	Phosphatidylcholine (acyl-alkyl) C38:0	2.351E-02	7.053E-02	1.295E+01	0	4.845 (1.377)	2.221 (0.402)	2.283 (0.396)
PC ae C38:1	Phosphatidylcholine (acyl-alkyl) C38:1	3.024E-02	9.072E-02	1.338E+01	0	0.912 (0.370)	-0.252 (0.599)	-0.134 (0.553)
PC ae C38:2	Phosphatidylcholine (acyl-alkyl) C38:2	3.385E-02	1.016E-01	2.719E+01	1	1.409 (0.678)	0.351 (0.634)	0.578 (0.620)
PC ae C38:3	Phosphatidylcholine (acyl-alkyl) C38:3	3.160E-02	9.481E-02	1.092E+01	0	6.974 (1.972)	2.746 (0.400)	2.859 (0.383)
PC ae C38:4	Phosphatidylcholine (acyl-alkyl) C38:4	8.331E-02	2.499E-01	1.028E+01	0	33.453 (8.574)	5.019 (0.362)	5.061 (0.329)
PC ae C38:5	Phosphatidylcholine (acyl-alkyl) C38:5	6.849E-02	2.055E-01	1.012E+01	0	32.118 (8.396)	4.959 (0.366)	4.952 (0.339)
PC ae C38:6	Phosphatidylcholine (acyl-alkyl) C38:6	3.802E-02	1.141E-01	1.040E+01	0	14.702 (4.036)	3.826 (0.387)	3.882 (0.382)
PC ae C40:1	Phosphatidylcholine (acyl-alkyl) C40:1	1.997E-02	5.992E-02	1.239E+01	0	3.221 (0.968)	1.624 (0.430)	1.560 (0.386)
PC ae C40:2	Phosphatidylcholine (acyl-alkyl) C40:2	2.122E-02	6.366E-02	1.034E+01	0	2.608 (0.690)	1.334 (0.376)	1.408 (0.351)
PC ae C40:3	Phosphatidylcholine (acyl-alkyl) C40:3	2.137E-02	6.412E-02	1.164E+01	0	2.692 (0.703)	1.383 (0.362)	1.452 (0.353)

PC ae C40:4	Phosphatidylcholine (acyl-alkyl) C40:4	2.092E-02	6.276E-02	1.144E+01	0	7.166 (2.092)	2.784 (0.404)	2.863 (0.368)
PC ae C40:5	Phosphatidylcholine (acyl-alkyl) C40:5	2.725E-02	8.174E-02	1.119E+01	0	6.748 (1.745)	2.709 (0.357)	2.738 (0.324)
PC ae C40:6	Phosphatidylcholine (acyl-alkyl) C40:6	2.407E-02	7.220E-02	1.190E+01	0	10.282 (3.228)	3.295 (0.438)	3.423 (0.412)
PC ae C42:0	Phosphatidylcholine (acyl-alkyl) C42:0	1.564E-02	4.691E-02	1.654E+01	0	0.884 (0.195)	-0.210 (0.304)	-0.222 (0.285)
PC ae C42:1	Phosphatidylcholine (acyl-alkyl) C42:1	1.749E-02	5.248E-02	1.611E+01	0	1.139 (0.273)	0.149 (0.331)	0.129 (0.294)
PC ae C42:2	Phosphatidylcholine (acyl-alkyl) C42:2	1.716E-02	5.147E-02	1.393E+01	0	1.128 (0.250)	0.141 (0.307)	0.134 (0.270)
PC ae C42:3	Phosphatidylcholine (acyl-alkyl) C42:3	1.612E-02	4.835E-02	1.326E+01	0	1.370 (0.361)	0.407 (0.363)	0.402 (0.349)
PC ae C42:4	Phosphatidylcholine (acyl-alkyl) C42:4	1.593E-02	4.780E-02	1.388E+01	0	1.432 (0.439)	0.458 (0.406)	0.509 (0.374)
PC ae C42:5	Phosphatidylcholine (acyl-alkyl) C42:5	2.101E-02	6.303E-02	1.243E+01	0	3.697 (1.019)	1.837 (0.370)	1.885 (0.348)
PC ae C44:3	Phosphatidylcholine (acyl-alkyl) C44:3	1.456E-02	4.368E-02	2.956E+01	0	1.520 (0.450)	0.551 (0.379)	0.489 (0.321)
PC ae C44:4	Phosphatidylcholine (acyl-alkyl) C44:4	1.547E-02	4.640E-02	1.936E+01	2	0.730 (0.202)	-0.504 (0.381)	-0.489 (0.330)
PC ae C44:5	Phosphatidylcholine (acyl-alkyl) C44:5	1.633E-02	4.899E-02	1.659E+01	0	2.400 (0.824)	1.191 (0.447)	1.209 (0.405)
PC ae C44:6	Phosphatidylcholine (acyl-alkyl) C44:6	1.579E-02	4.738E-02	1.827E+01	0	2.396 (0.819)	1.190 (0.441)	1.246 (0.401)
SM (OH) C14:1	Hydroxysphingomyelin C14:1	7.575E-02	2.272E-01	1.160E+01	0	3.125 (0.900)	1.589 (0.396)	1.716 (0.380)
SM (OH) C16:1	Hydroxysphingomyelin C16:1	5.317E-02	1.595E-01	1.303E+01	0	2.806 (0.748)	1.440 (0.375)	1.558 (0.357)
SM (OH) C22:1	Hydroxysphingomyelin C22:1	1.071E-01	3.212E-01	1.745E+01	0	5.537 (1.792)	2.401 (0.437)	2.556 (0.450)
SM (OH) C22:2	Hydroxysphingomyelin C22:2	9.794E-02	2.938E-01	1.946E+01	0	5.919 (1.920)	2.495 (0.449)	2.625 (0.445)
SM (OH) C24:1	Hydroxysphingomyelin C24:1	4.889E-02	1.467E-01	1.997E+01	0	1.249 (0.378)	0.257 (0.427)	0.410 (0.433)
SM C16:0	Sphingomyelin C16:0	8.948E-01	2.684E+00	1.477E+01	0	95.065 (21.952)	6.535 (0.322)	6.596 (0.303)
SM C16:1	Sphingomyelin C16:1	1.469E-01	4.406E-01	1.205E+01	0	16.389 (4.256)	3.988 (0.369)	4.041 (0.341)
SM C18:0	Sphingomyelin C18:0	2.891E-01	8.673E-01	1.414E+01	0	35.610 (8.680)	5.113 (0.343)	5.145 (0.324)
SM C18:1	Sphingomyelin C18:1	1.038E-01	3.113E-01	1.330E+01	0	23.458 (6.564)	4.498 (0.399)	4.521 (0.370)
SM C20:2	Sphingomyelin C20:2	4.827E-02	1.448E-01	2.404E+01	0	0.690 (0.208)	-0.596 (0.420)	-0.531 (0.406)
SM C22:3	Sphingomyelin C22:3	2.090E-01	6.270E-01	1.429E+01	5	1.638 (0.616)	0.603 (0.587)	0.479 (0.626)
SM C24:0	Sphingomyelin C24:0	1.519E-01	4.557E-01	1.577E+01	0	24.188 (5.912)	4.554 (0.348)	4.676 (0.325)
SM C24:1	Sphingomyelin C24:1	4.593E-01	1.378E+00	1.553E+01	0	51.386 (13.183)	5.639 (0.358)	5.699 (0.342)
SM C26:0	Sphingomyelin C26:0	3.911E-02	1.173E-01	2.985E+01	0	0.270 (0.076)	-1.946 (0.409)	-1.805 (0.371)
SM C26:1	Sphingomyelin C26:1	3.853E-02	1.156E-01	2.045E+01	0	0.631 (0.161)	-0.709 (0.360)	-0.595 (0.330)

Characteristics include LOD, LOQ, interassay CV, NAs, and mean and SD of original and log2-transformed metabolite concentrations of the total dataset and mean and SD of the log2-transformed metabolite concentrations of offspring of normal weight mothers (n = 111) as reference group. Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. CV, coefficient of variation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOD, limit of detection; LOQ: limit of quantification; NA, not available; SD, standard deviation; TG, triglyceride.

Table S 2: Abbreviations, full names and explanation and mean of metabolite sums and ratios

Abbreviations	Full name	Explanation	Mean (SD) total dataset	Mean (SD) total dataset, log2-transformed	Mean (SD) offspring of normal weight mothers, log2-transformed
Ratios					
Acyl-carnitines					
(AC C16:0 + AC C18:0) / AC C0	(Hexadecanoylcarnitine + Octadecanoylcarnitine) / free carnitine	Activity of carnitine palmitoyltransferase I	0.022 (0.011)	-5.682 (0.646)	-5.640 (0.654)
(AC C2:0 + AC C3:0) / AC C0	(Acetylcarnitine + propionylcarnitine) / free carnitine	Measure of overall β-oxidation activity	0.132 (0.030)	-2.951 (0.308)	-2.963 (0.314)
AC C12:0 / AC C10:0	Dodecanoylcarnitine / decanoylcarnitine	Activity of acyl-CoA dehydrogenase medium chain	0.774 (0.182)	-0.410 (0.347)	-0.370 (0.400)
AC C14:0 / AC C16:1	Tetradecanoylcarnitine / Hexadecenoylcarnitine	Activity of stearoyl-CoA desaturase	0.879 (0.165)	-0.211 (0.263)	-0.134 (0.287)
AC C2:0 / AC C0	Acetylcarnitine / free carnitine	Measure of β-oxidation activity of even numbered fatty acids	0.115 (0.028)	-3.160 (0.333)	-3.162 (0.333)
AC C3:0 / AC C4:0	Propionylcarnitine / butyrylcarnitine	Activity of acyl-CoA dehydrogenase short chain	7.072 (3.082)	2.692 (0.626)	2.623 (0.663)
Amino acids					
Asn / Gln	Asparagine /glutamine	Related to enzymatic activity of asparagine synthetase	Not used in main analysis	Not used in main analysis	Not used in main analysis
Asp /Asn	Aspartate /asparagine	Related to enzymatic activity of asparaginase	Not used in main analysis	Not used in main analysis	Not used in main analysis
Cit / Arg	Citrulline / arginine	Related to enzymatic activity of nitric oxide synthase	0.647 (0.478)	-0.908 (0.862)	-1.061 (0.822)
Cit / Orn	Citrulline / ornithine	Related to enzymatic activity of ornithine carbamoylphosphate transferase	0.079 (0.019)	-3.706 (0.340)	-3.754 (0.347)
Fisher	BCAA / aromatic acids	Indicator of liver damage	1.762 (0.285)	0.799 (0.232)	0.762 (0.219)
Glu/Gln	Glutamate / glutamine	Related to enzymatic activity of glutaminase	Not used in main analysis	Not used in main analysis	Not used in main analysis

Gly / PC ae C38:2	Glycine / phosphatidylcholine (acyl-alkyl) C38:2	Related to enzymatic activity of carbamoyl-phosphate synthase 1	330.426 (141.890)	8.242 (0.610)	8.126 (0.591)
Orn / Arg	Ornithine / arginine	Related to enzymatic activity of arginase	8.406 (5.806)	2.798 (0.871)	2.693 (0.851)
Orn / Ser	Ornithine / serine	Related to enzymatic activity of phosphoglycerate dehydrogenase	0.864 (0.312)	-0.286 (0.450)	-0.315 (0.446)
Tyr / Phe	Tyrosin / Phenylalanine	Related to enzymatic activity of phenylalanine hydroxylase	0.779 (0.120)	-0.377 (0.219)	-0.414 (0.200)
Bile acids					
Conj. BA / unconj. BA	Conjugated bile acids / unconjugated bile acids	conj.BAs/unconj.BAs	67.343 (60.352)	5.592 (1.213)	5.643 (1.180)
Prim. BA / sec. BA	Primary bile acids / secondary bile acids	prim.BAs/sec.BAs	68.481 (61.195)	5.617 (1.219)	5.620 (1.276)
Phospholipids					
LysoPC / PC	Lysophosphatidylcholine / phosphatidylcholines	Indicator of phospholipase activity	0.056 (0.013)	-4.209 (0.338)	-4.253 (0.302)
MUFA PC / SFA PC	Monounsaturated phosphatidalycholines / saturated phosphatidlycholines	Measure of the activity of fatty acid desaturases and indicator of nutritional lipid composition	7.859 (1.017)	2.962 (0.186)	2.933 (0.205)
PC aa C36:3 / PC aa C36:4	Phosphatidylcholine (diacyl) C36:3 / phosphatidylcholine (diacyl) C36:4	Related to enzymatic activity of fatty acid desaturase 1	0.377 (0.094)	-1.451 (0.354)	-1.380 (0.353)
PC aa C40:3 / PC aa C42:5	Phosphatidylcholine (diacyl) C40:3 / phosphatidylcholine (diacyl) C42:5	Related to enzymatic activity of ELOVL fatty acid elongase 2	1.479 (0.328)	0.528 (0.331)	0.525 (0.304)
PC ae C32:1 / PC ae C34:1	Phosphatidylcholine (acyl-alkyl) C32:1 / phosphatidylcholine (acyl-alkyl) C34:1	Related to enzymatic activity of pleckstrin homology, MyTH4 and FERM domain containing H1	0.398 (0.055)	-1.343 (0.199)	-1.343 (0.222)
PC ae C38:1 / PC aa C28:1	Phosphatidylcholine (acyl-alkyl) C38:1 / phosphatidylcholine (diacyl) C28:1	Related to enzymatic activity of sphingosine-1-phosphate phosphatase 1	1.077 (0.436)	0.001 (0.552)	0.044 (0.555)
PC ae C44:5 / PC ae C42:5	Phosphatidylcholine (acyl-alkyl) C44:5 / phosphatidylcholine (acyl-alkyl) C42:5	Related to enzymatic activity of electron transfer flavoprotein dehydrogenase	0.643 (0.069)	-0.646 (0.155)	-0.676 (0.139)
PUFA-PC / MUFA-PC	Polyunsaturated phosphatidylcholines / saturated phosphatidalycholines	Related to enzymatic activity of of fatty acid desaturases and indicator of nutritional lipid composition	5.059 (0.712)	2.325 (0.205)	2.351 (0.202)

PUFA-PC / SFA-PC	Polyunsaturated phosphatidylcholines / monounsaturated phosphatidalycholines	Related to enzymatic activity of fatty acid desaturases and indicator of nutritional lipid composition	39.331 (4.799)	5.287 (0.177)	5.284 (0.182)
SM (OH) C24:1 / SM C16:0	Hydroxysphingomyelin C24:1 / sphingomyelin C16:0	Related to enzymatic activity of of serine palmitoyltransferase long chain base subunit 3	0.013 (0.003)	-6.277 (0.319)	-6.186 (0.340)
Sums					
Acyl-carnitines					
AC C16:0 + AC C18:0	Hexadecanoylcarnitine + octadecanoylcarnitine	Sum for calculation of ratio	0.439 (0.219)	-1.341 (0.660)	-1.368 (0.633)
AC C2:0 + AC C3:0	Acetylcarnitine + propionylcarnitine	Sum for calculation of ratio	2.753 (0.946)	1.392 (0.430)	1.321 (0.440)
Carnitines	Carnitines	All carnitines	4.538 (1.388)	2.124 (0.400)	2.073 (0.387)
Even. carn	Even-chain carnitines	All carnitines with even chain	3.753 (1.218)	1.842 (0.426)	1.793 (0.412)
Long. carn	Long-chain carnitines	All metabolites starting with AC C16, AC C17, AC C18	0.777 (0.379)	-0.514 (0.650)	-0.547 (0.618)
Medium. carn	Medium-chain carnitines	All metabolites starting with AC C10, AC C11, AC C12, AC C13, AC C14	0.263 (0.115)	-2.042 (0.562)	-2.014 (0.516)
Odd. carn	Odd-chain carnitines	All carnitines with odd chain	0.658 (0.202)	-0.662 (0.397)	-0.714 (0.384)
Amino acids					
AA	Amino acids	All amino acids	4131.727 (526.935)	12.001 (0.180)	12.055 (0.199)
Arom. AA	Aromatic acids	Phe, Trp, Tyr	258.575 (34.661)	8.001 (0.195)	8.029 (0.181)
BCAA	Branched-chain amino acids	Ile, Leu, Val; Indicator of short-term metabolic control	453.430 (79.068)	8.803 (0.253)	8.790 (0.251)
Ess. AA	Essential amino acids	Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, His, Tyr; Indicator of nutritional status	1631.716 (199.923)	10.661 (0.176)	10.678 (0.196)
Glucog. AA	Glucogenic amino acids	Ala, Gly, Ser; Indicator of balance between glycolysis and glyconeogenese	1272.272 (255.863)	10.284 (0.289)	10.381 (0.292)
Noness. AA	Non-essential amino acids	Ala, Arg, Asn, Gln, Glu, Gly, Pro, Ser; Indicator of metabolic/catabolic state	2.283.298 (357.905)	11.140 (0.222)	11.220 (0.235)
Bile acids					
Conj. BA	Conjugated bile acids	GCA, GCDCA, TCA, TCDCA, GDCA, GLCA, TDCA, TLCA, GUDCA, TUDCA	1947.646 (1350.808)	10.666 (0.859)	10.655 (0.978)
Prim. BA	Primary bile acids	CA, CDCA, GCA, GCDCA, TCA, TCDCA	1960.440 (1289.442)	10.691 (0.839)	10.678 (0.963)

Sec. BA	Secondary bile acids	DCA, LCA, GDCA, GLCA, TDCA, TLCA, UDCA, GUDCA, TUDCA	46.137 (60.321)	5.040 (1.143)	5.007 (1.150)
Unconj. BA	Unconjugated bile acids	CA, CDCA, DCA, LCA, UDCA	74.635 (64.016)	5.940 (0.840)	5.844 (0.907)
Phospholipids					
Long. PC	Long-chain phosphatidylcholines (acyl-alkyl)	All long. PC aa + long. PC ae	191.436 (54.309)	7.526 (0.393)	7.607 (0.365)
Long. PC aa	Long-chain phosphatidylcholines (diacyl)	All PC aa containing long fatty acid chains (C40, C42, C44)	142.015 (44.739)	7.083 (0.436)	7.174 (0.407)
Long. PC ae	Long-chain phosphatidylcholines	All PC ae containing long fatty acid chains (C40, C42, C44)	49.421 (12.286)	5.586 (0.343)	5.636 (0.313)
Long. SM	Long-chain sphingomyelins	All long. SM C + long SM (OH)	77.724 (18.848)	6.240 (0.340)	6.322 (0.322)
Long. SM C	Long-chain non-hydroxysphingomyelins	All SM containing a long fatty acid chain (C24, C26)	76.475 (18.578)	6.216 (0.341)	6.297 (0.323)
Long. SM OH	Long-chain hydroxysphingomyelins	All SM containing an OH containing a long fatty acid chain (C24, C26)	1.249 (0.378)	0.257 (0.427)	0.410 (0.433)
LysoPC	Lysophosphatidylcholines (acyl)	All LysoPC	177.915 (40.680)	7.439 (0.325)	7.408 (0.308)
Mono. PC	Monounsaturated phosphatidylcholines	Mono. PC aa + mono. PC ae	540.789 (142.871)	9.032 (0.366)	9.032 (0.341)
Mono. PC aa	Monounsaturated phosphatidylcholines (diacyl)	All PC aa containing exactly 1 double bond in the fatty acid chains (:1)	506.503 (134.600)	8.937 (0.368)	8.935 (0.344)
Mono. PC ae	Monounsaturated phosphatidylcholines (acyl-alkyl)	All PC ae containing exactly 1 double bond in the fatty acid chains (:1)	34.286 (9.151)	5.051 (0.373)	5.082 (0.344)
PC	Phosphatidylcholines	All PC	3299.838 (761.666)	11.652 (0.324)	11.674 (0.309)
PC aa	Phosphatidylcholines (diacyl)	All PC aa	3026.550 (699.148)	11.527 (0.324)	11.548 (0.310)
PC ae	Phosphatidylcholines (acyl-alkyl)	All PC ae	273.288 (67.785)	8.052 (0.347)	8.092 (0.324)
Poly. PC	Polyunsaturated phosphatidylcholines (acyl-alkyl)	Poly. PC aa + poly. PC ae	2689.689 (622.739)	11.356 (0.326)	11.383 (0.313)
Poly. PC aa	Polyunsaturated phosphatidylcholines (diacyl)	All PC aa containing >1 double bond in the fatty acid chains	2461.772 (570.680)	11.228 (0.326)	11.254 (0.315)
Poly. PC ae	Polyunsaturated phosphatidylcholines	All PC ae containing >1 double bond in the fatty acid chains	227.917 (56.833)	7.790 (0.349)	7.830 (0.326)

Sat. LysoPC	Saturated lysophosphatidylcholines	All LysoPC containing a fatty acid chain without double bond	133.561 (33.004)	7.019 (0.352)	7.001 (0.338)
Sat. PC	Saturated PCs	Sat. PC aa + sat. PC ae	69.360 (17.995)	6.069 (0.366)	6.099 (0.350)
Sat. PC aa	Saturated phosphatidylcholines (diacyl)	All PC aa containing no double bond in the fatty acid chains (:0)	58.275 (15.770)	5.814 (0.380)	5.836 (0.367)
Sat. PC ae	Saturated phosphatidylcholines (acyl-alkyl)	All PC ae containing no double bond in the fatty acid chains (:0)	11.085 (2.836)	3.425 (0.364)	3.493 (0.347)
Saturmono. PC	Saturated and monounsaturated phosphatodylcholines	Mono. PC + sat. PC	540.789 (142.871)	9.032 (0.366)	9.032 (0.341)
Short. PC	Short-chain PCs	All short. PC aa + short. PC ae	811.522 (223.120)	9.613 (0.387)	9.627 (0.351)
Short. PC aa	Short-chain phosphatidylcholines (acyl-alkyl)	All PC aa containing shorter fatty acid chains (C26 - C34)	773.451 (213.369)	9.543 (0.388)	9.556 (0.353)
Short. PC ae	Short-chain phosphatidylcholines (diacyl)	All PC ae containing shorter fatty acid chains (C26 - C34)	38.242 (11.198)	5.201 (0.398)	5.245 (0.382)
SM	Sphingomyelins	All SM	267.943 (62.736)	8.028 (0.328)	8.091 (0.312)
SM (OH)	Hydroxysphingomyelins	All SM containing an OH group	18.637 (5.437)	4.163 (0.402)	4.300 (0.401)
SM C	Sphingomyelins	All SM not containing an OH group	249.306 (58.126)	7.924 (0.327)	7.981 (0.310)
Unsat. lysoPC	Unsaturated lysophosphatidylcholines	All LysoPC containing a fatty acid chain with at least one double bond	44.354 (11.400)	5.425 (0.366)	5.356 (0.340)
Very. lysoPC	Very long-chain lysophosphatidylcholines	All LysoPC containing a very long fatty acid chain (C24, C26, C28)	1.542 (0.191)	0.614 (0.171)	0.596 (0.162)

Abbreviations, full names and explanations of ratios and sums. Mean and SD of original and log2-transformed metabolite concentrations of the total dataset and mean and SD of the log2-transformed metabolite concentrations of offspring of normal weight mothers (n = 111) as reference group are provided. Bile acids are given in ng/mL, all other metabolites in µmol/L. SD, standard deviation. Ratios are provided according to (185-193, 243)

Table S 3: Beta-estimates (95% CI) for metabolite concentrations, ratios and sums in female UCB compared to female adult blood

	female UCB vs. female adult blood
Metabolites	β-estimate (95% CI)
Acyl-carnitines	
AC 2-M-C3:0	not significant
AC 2-M-C4:0	0.558 (0.275; 0.840)***
AC 3-M-C4:0	not significant
AC C0	-0.838 (-0.990; -0.687)***
AC C10:0	-2.753 (-3.029; -2.477)***
AC C10:1	-1.883 (-2.128; -1.638)***
AC C12:0	-1.740 (-2.061; -1.419)***
AC C12:1	-1.849 (-2.167; -1.530)***
AC C14:0	0.465 (0.163; 0.767)**
AC C16:0	1.030 (0.753; 1.308)***
AC C16:1	0.745 (0.395; 1.094)***
AC C18:0	not significant
AC C18:1	not significant
AC C18:2	1.177 (0.853; 1.501)***
AC C2:0	-0.361 (-0.578; -0.143)**
AC C3-DC	0.303 (0.115; 0.491)**
AC C3-M-DC	not significant
AC C3:0	0.347 (0.112; 0.582)**
AC C4:0	not significant
AC C4-DC	0.648 (0.419; 0.877)***
AC C4-OHa	1.020 (0.677; 1.363)***
AC C4-OHb	not significant
AC C5:1	-0.524 (-0.791; -0.258)***
AC C5-DC	not significant
AC C6:0	not significant
AC C6-DC	1.012 (0.607; 1.417)***
AC C6-OHa	0.595 (0.292; 0.898)***
AC C6-OHb	not significant
AC C7-DC	not significant
AC C8:0	-2.004 (-2.263; -1.745)***
AC C8:1	-1.122 (-1.544; -0.701)***
AC iso-C11:0	-1.968 (-2.320; -1.616)***
AC iso-C13:0	-0.816 (-1.231; -0.401)***
AC iso-C15:0	not significant
AC iso-C9:0	-1.499 (-1.938; -1.061)***
Amino acids	
1-M-His	0.397 (0.174; 0.620)***
3-M-His	not significant
AAB	not significant
Ala	1.014 (0.850; 1.179)***

Arg	-1.232 (-1.558; -0.906)***
Asp	2.584 (2.399; 2.769)***
BAIB	not significant
Cit	-0.822 (-1.000; -0.645)***
Gln	-0.199 (-0.321; -0.077)**
Glu	3.225 (3.079; 3.372)***
Gly	0.773 (0.625; 0.920)***
His	0.871 (0.737; 1.006)***
Ile	0.705 (0.599; 0.850)***
Leu	0.353 (0.214; 0.493)***
Lys	1.305 (1.189; 1.421)***
Met	1.001 (0.895; 1.170)***
OH-Pro	1.650 (1.449; 1.851)***
Orn	1.664 (1.516; 1.812)***
Phe	0.912 (0.825; 0.999)***
Pro	0.457 (0.333; 0.580)***
Ser	0.896 (0.765; 1.027)***
Thr	1.345 (1.150; 1.541)***
Trp	0.419 (0.297; 0.541)***
Tyr	0.530 (0.415; 0.645)***
Val	0.316 (0.209; 0.422)***
β-Ala	-0.609 (-1.024; 0.193)**
Ratios	
Acyl-carnitines	
AC C12:0/AC C10:0	1.013 (0.826; 1.200)***
(AC C16:0+AC C18:0)/AC C0	1.761 (1.455; 2.068)***
(AC C2:0+AC C3:0)/AC C0	0.502 (0.337; 0.667)***
AC C14:0/AC C16:1	-0.273 (-0.441; 0.106)**
AC C2:0/AC C0	0.466 (0.288; 0.643)***
AC C3:0/AC C4:0	not significant
Amino acids	
Asn/Gln	1.295 (1.150; 1.440)***
Asp/Asn	1.515 (1.346; 1.684)***
Cit/Arg	0.384 (0.038; 0.731)*
Cit/Orn	-2.507 (-2.669; -2.344)***
Fisher ratio	-0.265 (-0.374; -0.156)***
Glu/Gln	3.440 (3.244; 1.637)***
Orn/Arg	2.851 (2.478; 3.223)***
Orn/Ser	0.768 (0.631; 0.905)***
Tyr/Phe	0.362 (-0.471; -0.252)***
Sums	
Acyl-carnitines	
AC	-0.765 (-0.909; -0.620)***
AC C16:0+AC C18:0	0.926 (0.654; 1.198)***
AC C2:0+AC C3:0	-0.323 (-0.528; 0.118)**

Even. AC	-0.319 (-0.508; -0.129)**
Long. AC	0.740 (0.469; 1.012)***
Medium. AC	-1.649 (-1.918; -1.380)***
Odd. AC	not significant
Amino acids	
AA	0.831 (0.747; 0.914)***
Aromatic AA	0.649 (0.567; 0.730)***
BCAA	0.414 (0.303; 0.525)***
Essential AA	0.882 (0.796; 0.967)***
Glucogenic AA	0.927 (0.803; 1.050)***
Nonessential AA	0.736 (0.636; 0.836)***

Data are presented as β-estimate (95% CI) of acyl-carnitine and amino acid concentrations (µmol/L) in female UCB (n = 60) compared to female adult blood (n = 20). Data are based on univariate linear regression models. Statistical significance is based on p-values adjusted for multiple testing by the method of Benjamini-Hochberg. * = q-value < 0.05, ** = q-value < 0.01 *** = q-value < 0.001. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. AA, amino acids; AC, acyl-carnitines; CI, confidence interval; UCB, umbilical cord blood.

Table S 4: p-values of the pre- and perinatal factors tested in a multiple linear regression model for their influence on UCB amino acid and acyl-carnitine concentrations

Metabolites	Offspring sex	GA	Birth weight	Mode of dlivery	GWG excessive	GWG inadequate	Maternal HbA1c at delivery
Acyl-carnitines							
AC 2-M-C3:0	1.00E+00	1.00E+00	4.56E-01	1.00E+00	9.58E-01	6.78E-01	3.29E-01
AC 2-M-C4:0	1.00E+00	4.41E-01	1.00E+00	9.29E-01	9.99E-01	1.00E+00	1.00E+00
AC 3-M-C4:0	1.40E-01	5.60E-01	1.00E+00	1.00E+00	9.98E-01	8.72E-01	8.59E-01
AC C0	9.25E-01	1.00E+00	5.41E-01	1.00E+00	1.00E+00	9.33E-01	1.00E+00
AC C10:0	1.00E+00	9.86E-01	1.00E+00	4.64E-01	8.85E-01	9.64E-01	1.00E+00
AC C10:1	8.46E-01	8.15E-01	9.81E-01	7.57E-01	8.65E-01	9.99E-01	9.99E-01
AC C12:0	1.00E+00	8.94E-01	1.00E+00	1.53E-01	6.51E-01	1.00E+00	9.99E-01
AC C12:1	1.00E+00	7.13E-01	9.99E-01	6.23E-01	6.15E-01	8.52E-01	9.80E-01
AC C14:0	9.95E-01	9.85E-01	7.16E-01	4.06E-01	1.00E+00	9.92E-01	9.17E-01
AC C16:0	9.99E-01	9.83E-01	8.65E-01	7.12E-01	1.00E+00	8.52E-01	9.98E-01
AC C16:1	9.94E-01	6.94E-01	8.47E-01	8.59E-01	1.00E+00	1.00E+00	8.17E-01
AC C18:0	1.00E+00	1.00E+00	9.98E-01	5.71E-01	1.00E+00	6.49E-01	1.00E+00
AC C18:1	9.97E-01	9.99E-01	6.96E-01	6.82E-01	9.99E-01	1.00E+00	1.00E+00
AC C18:2	5.51E-01	5.94E-01	1.00E+00	7.17E-01	1.00E+00	8.78E-01	9.86E-01
AC C2:0	1.00E+00	1.00E+00	1.00E+00	8.39E-01	7.01E-01	9.17E-01	1.00E+00
AC C3-DC	1.00E+00	1.00E+00	9.96E-01	7.00E-01	7.58E-01	9.98E-01	9.37E-01
AC C3-M-DC	9.04E-01	1.00E+00	1.00E+00	9.98E-01	9.98E-01	9.99E-01	9.11E-01
AC C3:0	7.95E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	9.96E-01
AC C4:0	1.00E+00	3.61E-01	1.00E+00	1.00E+00	8.34E-01	1.00E+00	1.00E+00
AC C4-DC	1.00E+00	1.00E+00	9.98E-01	9.54E-01	7.52E-01	1.00E+00	3.76E-01
AC C4-OHa	1.00E+00	9.99E-01	9.71E-01	9.99E-01	1.66E-01	2.84E-01	1.00E+00
AC C4-OHb	1.00E+00	9.96E-01	8.86E-01	1.00E+00	1.00E+00	2.81E-01	1.00E+00
AC C5:1	1.00E+00	9.98E-01	1.00E+00	1.00E+00	9.78E-01	7.53E-01	1.00E+00
AC C5-DC	7.87E-01	9.91E-01	1.00E+00	1.00E+00	1.00E+00	9.70E-01	9.92E-01
AC C6:0	4.10E-01	9.99E-01	9.20E-01	9.99E-01	5.29E-01	6.52E-01	1.00E+00
AC C6-DC	1.00E+00	8.82E-01	9.20E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00
AC C6-OHa	1.00E+00	5.31E-01	1.00E+00	1.00E+00	1.50E-01	7.28E-01	9.50E-01
AC C6-OHb	1.00E+00	9.85E-01	1.00E+00	1.00E+00	1.63E-01	9.88E-01	1.00E+00
AC C7-DC	9.82E-01	9.94E-01	8.84E-01	1.00E+00	9.99E-01	1.00E+00	1.00E+00
AC C8:0	4.01E-01	9.93E-01	1.00E+00	4.95E-01	9.53E-01	9.85E-01	1.00E+00
AC C8:1	9.99E-01	1.00E+00	9.99E-01	9.32E-01	2.71E-01	9.30E-01	1.33E-01
AC iso-C11:0	3.79E-01	7.13E-02	7.09E-01	7.05E-01	1.00E+00	1.00E+00	1.13E-01
AC iso-C13:0	6.32E-01	1.00E+00	1.00E+00	2.63E-01	1.00E+00	1.00E+00	1.00E+00
AC iso-C15:0	1.83E-01	5.00E-01	5.46E-01	9.96E-01	1.00E+00	9.67E-01	1.00E+00
AC iso-C9:0	1.00E+00	7.20E-01	9.68E-01	4.77E-01	9.56E-01	9.97E-01	1.00E+00
Amino acids							
1-M-His	1.00E+00	9.57E-01	1.00E+00	1.00E+00	9.78E-01	1.00E+00	9.21E-01
3-M-His	1.00E+00	9.90E-01	1.00E+00	7.11E-01	1.00E+00	1.00E+00	8.33E-01
AAB	1.00E+00	9.66E-01	1.00E+00	9.96E-01	9.97E-01	1.00E+00	1.00E+00
Ala	9.37E-01	8.38E-01	9.51E-01	9.90E-03	9.53E-01	9.78E-01	9.24E-01
Arg	9.39E-01	9.31E-01	9.64E-01	1.00E+00	9.88E-01	9.95E-01	1.00E+00
Asp	9.93E-01	9.94E-01	9.93E-01	9.95E-01	1.00E+00	1.00E+00	9.65E-01
BAIB	9.91E-01	1.00E+00	9.95E-01	1.00E+00	9.97E-01	1.00E+00	9.95E-01
Cit	1.00E+00	9.93E-01	9.29E-01	7.69E-01	1.00E+00	9.62E-01	9.79E-01
Gln	2.18E-01	1.00E+00	5.67E-01	1.00E+00	9.98E-01	9.99E-01	1.00E+00
Glu	2.97E-01	9.76E-01	9.99E-01	9.22E-01	9.96E-01	8.26E-01	9.82E-01
Gly	1.00E+00	1.00E+00	4.08E-01	9.89E-01	1.00E+00	1.00E+00	9.83E-01
His	9.73E-01	1.00E+00	9.83E-01	8.83E-01	1.00E+00	9.44E-01	8.41E-01
Ile	1.00E+00	1.00E+00	9.16E-01	1.31E-01	1.00E+00	9.98E-01	7.83E-01
Leu	1.00E+00	1.00E+00	1.00E+00	8.84E-02	1.00E+00	1.00E+00	9.93E-01
Lys	1.00E+00	1.00E+00	9.96E-01	2.09E-01	1.00E+00	3.21E-01	1.00E+00
Met	9.97E-01	9.90E-01	9.94E-01	2.00E-03	1.00E+00	8.65E-01	9.22E-01
OH-Pro	5.81E-01	<1.00E-03	8.95E-01	1.00E+00	2.75E-01	1.00E+00	1.00E+00
Orn	9.89E-01	1.00E+00	4.47E-01	2.70E-03	1.00E+00	4.62E-01	1.00E+00
Phe	5.99E-01	9.94E-01	9.84E-01	2.10E-03	1.00E+00	9.98E-01	7.44E-01
Pro	9.69E-01	1.00E+00	8.85E-01	6.20E-03	8.62E-01	1.00E+00	9.94E-01
Ser	9.94E-01	1.00E+00	1.00E+00	6.02E-02	1.00E+00	9.63E-01	1.00E+00
Thr	7.99E-01	9.96E-01	8.94E-01	6.95E-01	1.00E+00	1.00E+00	2.66E-01
Trp	1.00E+00	1.41E-01	1.00E+00	1.00E+00	1.00E+00	9.90E-01	1.00E+00
Tyr	1.00E+00	9.99E-01	1.00E+00	1.57E-01	1.00E+00	8.94E-01	9.99E-01

Val	1.00E+00	1.00E+00	1.00E+00	1.28E-01	1.00E+00	1.00E+00	1.00E+00
β-Ala	1.00E+00	1.00E+00	1.00E+00	8.22E-01	6.51E-01	1.00E+00	8.19E-01

Data are p-values of multiple linear regression models based on n = 112 UCB samples with the categorical variables offspring sex (male/ female), mode of delivery (C-section/vaginal), GWG excessive (excessive/adequate), GWG inadequate (inadequate/adequate), and high maternal HbA1c (%) at delivery (< 5.7 % vs. ≥ 5.7 %), and the continuous variables GA and birth weight as explanatory variables and the UCB metabolite, amino acids and acyl-carnitines, as outcome variable. Offspring with missing data on any of the potential confounders were excluded from the analysis. Metabolite concentrations are µmol/L. A full list of metabolite abbreviations is provided in Table S 1. GA, gestational age; GWG, gestational weight gain, HbA1c, glycated hemoglobin

Table S 5: Association of maternal preconception obesity and UCB metabolites of both sexes

	Offspring of obese vs. offspring of normal weight mothers			Offspring of severely obese vs. offspring of normal weight mothers		
Metabolite	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Acyl-carnitines						
AC 2-M-C3:0	0.159 (0.073)	2.98E-02	2.23E-01	0.280 (0.076)	2.68E-04	**3.56E-03**
AC 2-M-C3:1	0.102 (0.101)	3.11E-01	4.35E-01	0.220 (0.105)	3.63E-02	7.52E-02
AC 2-M-C4:0	0.143 (0.078)	6.76E-02	2.55E-01	0.296 (0.081)	3.11E-04	**3.71E-03**
AC 3-M-C4:0	0.241 (0.137)	7.89E-02	2.79E-01	0.362 (0.142)	1.14E-02	**3.78E-02**
AC C0	0.089 (0.040)	2.42E-02	2.23E-01	0.114 (0.041)	5.70E-03	**2.46E-02**
AC C10	0.081 (0.073)	2.68E-01	4.23E-01	0.108 (0.076)	1.57E-01	1.69E-01
AC C10:1	0.078 (0.064)	2.20E-01	4.13E-01	0.119 (0.066)	7.18E-02	1.08E-01
AC C11:0	0.104 (0.082)	2.07E-01	4.09E-01	0.048 (0.085)	5.71E-01	3.26E-01
AC C12:0	0.052 (0.076)	4.90E-01	5.36E-01	0.082 (0.079)	3.02E-01	2.46E-01
AC C12:1	0.060 (0.076)	4.31E-01	5.04E-01	0.108 (0.079)	1.75E-01	1.78E-01
AC C13:0	-0.002 (0.079)	9.80E-01	6.53E-01	-0.039 (0.083)	6.41E-01	3.43E-01
AC C14:0	0.043 (0.086)	6.17E-01	5.81E-01	0.022 (0.090)	8.05E-01	3.74E-01
AC C14:1	0.103 (0.084)	2.19E-01	4.13E-01	0.137 (0.087)	1.17E-01	1.42E-01
AC C15:0	0.038 (0.092)	6.78E-01	5.89E-01	-0.039 (0.096)	6.82E-01	3.53E-01
AC C16:0	0.111 (0.084)	1.88E-01	3.99E-01	0.070 (0.088)	4.24E-01	2.87E-01
AC C16:1	0.118 (0.091)	1.93E-01	3.99E-01	0.216 (0.094)	2.28E-02	6.06E-02
AC C17:0	0.030 (0.079)	7.08E-01	5.89E-01	-0.039 (0.082)	6.37E-01	3.43E-01
AC C18:0	0.061 (0.071)	3.92E-01	4.86E-01	0.038 (0.074)	6.06E-01	3.40E-01
AC C18:1	0.123 (0.081)	1.29E-01	3.70E-01	0.198 (0.084)	1.89E-02	5.25E-02
AC C18:2	0.092 (0.091)	3.10E-01	4.35E-01	0.137 (0.095)	1.49E-01	1.62E-01
AC C2:0	0.061 (0.059)	3.09E-01	4.35E-01	0.102 (0.062)	1.00E-01	1.31E-01
AC C3:0	0.118 (0.071)	9.77E-02	3.21E-01	0.289 (0.074)	1.02E-04	**2.04E-03**
AC C3-DC	0.010 (0.049)	8.38E-01	6.15E-01	0.061 (0.051)	2.37E-01	2.15E-01
AC C3-M-DC	-0.053 (0.052)	3.10E-01	4.35E-01	-0.034 (0.054)	5.34E-01	3.13E-01
AC C4:0	0.091 (0.073)	2.13E-01	4.13E-01	0.116 (0.076)	1.26E-01	1.47E-01
AC C4:1	0.200 (0.171)	2.41E-01	4.23E-01	0.394 (0.178)	2.78E-02	6.79E-02
AC C4-DC	-0.010 (0.073)	8.95E-01	6.37E-01	-0.004 (0.077)	9.60E-01	4.11E-01
AC C4-OHa	0.080 (0.105)	4.51E-01	5.22E-01	0.164 (0.110)	1.36E-01	1.50E-01
AC C4-OHb	0.112 (0.152)	4.61E-01	5.29E-01	0.341 (0.158)	3.14E-02	7.09E-02
AC C5:1	0.162 (0.078)	3.70E-02	2.23E-01	0.248 (0.081)	2.26E-03	**1.42E-02**
AC C5-DC	0.086 (0.062)	1.66E-01	3.98E-01	0.112 (0.064)	8.18E-02	1.13E-01
AC C5-M-DC	-0.711 (0.146)	1.61E-06	**3.02E-04**	-0.575 (0.152)	1.75E-04	**2.62E-03**
AC C5-OH	0.129 (0.063)	4.25E-02	2.33E-01	0.250 (0.066)	1.62E-04	**2.62E-03**
AC C6:0	0.232 (0.098)	1.79E-02	2.23E-01	0.251 (0.102)	1.39E-02	**4.50E-02**
AC C6:1	0.037 (0.084)	6.62E-01	5.89E-01	0.015 (0.088)	8.62E-01	3.82E-01
AC C6-DC	0.139 (0.124)	2.65E-01	4.23E-01	0.336 (0.129)	9.68E-03	**3.31E-02**
AC C6-OHa	0.188 (0.092)	4.12E-02	2.33E-01	0.301 (0.096)	1.77E-03	**1.25E-02**
AC C6-OHb	0.152 (0.078)	5.16E-02	2.39E-01	0.217 (0.081)	7.71E-03	**2.88E-02**
AC C7:0	0.128 (0.073)	8.04E-02	2.79E-01	0.047 (0.076)	5.34E-01	3.13E-01
AC C7-DC	-0.107 (0.074)	1.52E-01	3.95E-01	-0.026 (0.077)	7.32E-01	3.65E-01

AC C8:0	0.116 (0.075)	1.27E-01	3.70E-01	0.138 (0.079)	8.02E-02	1.13E-01
AC C8:1	0.269 (0.107)	1.20E-02	1.96E-01	0.393 (0.111)	4.46E-04	**4.45E-03**
AC C8-OH	0.043 (0.069)	5.31E-01	5.49E-01	0.049 (0.072)	4.95E-01	3.06E-01
AC C9:0	-0.071 (0.094)	4.51E-01	5.22E-01	-0.188 (0.098)	5.55E-02	9.21E-02
AC iso-C11:0	0.017 (0.082)	8.37E-01	6.15E-01	0.034 (0.085)	6.90E-01	3.53E-01
AC iso-C13:0	-0.218 (0.100)	2.93E-02	2.23E-01	-0.281 (0.104)	7.09E-03	**2.73E-02**
AC iso-C15:0	-0.003 (0.079)	9.73E-01	6.52E-01	-0.081 (0.082)	3.25E-01	2.54E-01
AC iso-C17:0	0.033 (0.083)	6.92E-01	5.89E-01	-0.027 (0.087)	7.58E-01	3.71E-01
AC iso-C9:0	-0.056 (0.095)	5.54E-01	5.63E-01	-0.077 (0.099)	4.34E-01	2.87E-01
Amino acids						
1-M-His	-0.021 (0.058)	7.15E-01	5.89E-01	0.110 (0.060)	6.90E-02	1.07E-01
3-M-His	0.287 (0.257)	2.66E-01	4.23E-01	0.596 (0.267)	2.61E-02	6.50E-02
AAB	0.026 (0.070)	7.04E-01	5.89E-01	0.077 (0.072)	2.88E-01	2.44E-01
AADP	0.079 (0.062)	2.03E-01	4.04E-01	0.084 (0.065)	1.95E-01	1.91E-01
Ala	-0.004 (0.041)	9.28E-01	6.42E-01	-0.060 (0.043)	1.68E-01	1.73E-01
Anserine	-0.107 (0.230)	6.42E-01	5.89E-01	-0.005 (0.239)	9.83E-01	4.17E-01
Arg	-0.191 (0.097)	5.05E-02	2.39E-01	-0.412 (0.101)	5.62E-05	**1.35E-03**
Asn	-0.098 (0.039)	1.25E-02	1.96E-01	-0.135 (0.041)	9.78E-04	**7.80E-03**
Asp	-0.135 (0.052)	1.06E-02	1.96E-01	-0.169 (0.055)	2.08E-03	**1.38E-02**
BAIB	-0.131 (0.118)	2.67E-01	4.23E-01	-0.037 (0.122)	7.65E-01	3.71E-01
Carnosine	-0.118 (0.194)	5.45E-01	5.58E-01	-0.387 (0.200)	5.39E-02	9.08E-02
Cit	-0.054 (0.042)	1.94E-01	3.99E-01	-0.032 (0.043)	4.62E-01	2.92E-01
Cys-Cys	-0.111 (0.058)	5.87E-02	2.39E-01	0.040 (0.061)	5.15E-01	3.09E-01
Dimethylglycine	0.032 (0.063)	6.08E-01	5.81E-01	0.085 (0.066)	2.01E-01	1.94E-01
GABA	-0.116 (0.101)	2.52E-01	4.23E-01	-0.161 (0.104)	1.23E-01	1.44E-01
Gln	-0.020 (0.043)	6.36E-01	5.89E-01	-0.021 (0.044)	6.29E-01	3.43E-01
Glu	-0.061 (0.043)	1.61E-01	3.95E-01	-0.098 (0.045)	2.90E-02	6.80E-02
Gly	-0.075 (0.033)	2.19E-02	2.23E-01	-0.138 (0.034)	5.64E-05	**1.35E-03**
Hcys	0.119 (0.103)	2.49E-01	4.23E-01	0.161 (0.107)	1.35E-01	1.50E-01
His	-0.099 (0.037)	7.04E-03	1.96E-01	-0.241 (0.038)	7.04E-10	**8.42E-08**
Ile	0.044 (0.043)	3.04E-01	4.35E-01	0.092 (0.045)	4.15E-02	7.87E-02
Leu	0.031 (0.044)	4.80E-01	5.32E-01	0.036 (0.046)	4.29E-01	2.87E-01
Lys	0.003 (0.030)	9.29E-01	6.42E-01	-0.004 (0.031)	8.92E-01	3.90E-01
Met	-0.041 (0.028)	1.55E-01	3.95E-01	-0.081 (0.030)	6.44E-03	**2.56E-02**
OH-Pro	0.110 (0.032)	7.26E-04	**4.53E-02**	0.119 (0.033)	4.16E-04	**4.45E-03**
Orn	-0.028 (0.034)	4.09E-01	4.95E-01	-0.074 (0.035)	3.72E-02	7.54E-02
Phe	0.014 (0.027)	5.88E-01	5.81E-01	-0.005 (0.028)	8.55E-01	3.81E-01
Pro	-0.013 (0.026)	6.14E-01	5.81E-01	-0.033 (0.027)	2.29E-01	2.11E-01
Sarcosine	0.054 (0.055)	3.26E-01	4.44E-01	0.049 (0.057)	3.93E-01	2.75E-01
Ser	-0.105 (0.039)	7.88E-03	1.96E-01	-0.169 (0.041)	4.21E-05	**1.35E-03**
Thr	-0.004 (0.041)	9.23E-01	6.42E-01	-0.042 (0.042)	3.21E-01	2.54E-01
Trp	0.012 (0.034)	7.30E-01	5.89E-01	0.046 (0.035)	1.84E-01	1.83E-01
Tyr	0.041 (0.029)	1.57E-01	3.95E-01	0.022 (0.030)	4.69E-01	2.95E-01
Val	0.067 (0.030)	2.57E-02	2.23E-01	0.086 (0.031)	5.84E-03	**2.46E-02**
β-Ala	0.043 (0.088)	6.29E-01	5.86E-01	0.070 (0.092)	4.44E-01	2.87E-01

Amino acid derivatives						
Betaine	0.100 (0.046)	3.09E-02	2.23E-01	0.135 (0.048)	5.54E-03	**2.46E-02**
Choline	0.020 (0.041)	6.21E-01	5.81E-01	-0.013 (0.043)	7.67E-01	3.71E-01
Bile acids						
CA	-0.178 (0.135)	1.87E-01	3.99E-01	-0.042 (0.140)	7.65E-01	3.71E-01
CDCA	0.096 (0.080)	2.28E-01	4.13E-01	0.158 (0.083)	5.85E-02	9.59E-02
DCA	0.089 (0.221)	6.89E-01	5.89E-01	0.220 (0.229)	3.37E-01	2.56E-01
GCA	-0.189 (0.144)	1.91E-01	3.99E-01	-0.318 (0.150)	3.46E-02	7.52E-02
GCDCA	-0.101 (0.141)	4.75E-01	5.32E-01	0.027 (0.147)	8.55E-01	3.81E-01
GDCA	-0.293 (0.201)	1.45E-01	3.94E-01	-0.185 (0.209)	3.75E-01	2.68E-01
GLCA	-0.548 (0.293)	6.34E-02	2.52E-01	-0.622 (0.307)	4.46E-02	8.01E-02
GUDCA	-0.055 (0.192)	7.74E-01	6.06E-01	0.136 (0.200)	4.97E-01	3.06E-01
LCA	0.129 (0.296)	6.63E-01	5.89E-01	0.390 (0.302)	1.97E-01	1.92E-01
TCA	-0.123 (0.145)	3.98E-01	4.90E-01	-0.084 (0.151)	5.76E-01	3.28E-01
TCDCA	-0.049 (0.125)	6.92E-01	5.89E-01	0.060 (0.130)	6.42E-01	3.43E-01
TDCA	-0.208 (0.222)	3.50E-01	4.55E-01	-0.143 (0.227)	5.28E-01	3.13E-01
TLCA	-0.339 (0.204)	9.69E-02	3.21E-01	-0.193 (0.208)	3.54E-01	2.62E-01
TLCA-S	-0.464 (0.123)	1.88E-04	**1.76E-02**	-0.305 (0.128)	1.75E-02	5.10E-02
TUDCA	-0.202 (0.123)	1.00E-01	3.22E-01	0.004 (0.127)	9.73E-01	4.14E-01
UDCA	0.056 (0.222)	8.00E-01	6.08E-01	0.066 (0.226)	7.69E-01	3.71E-01
Lipids						
HDL cholesterol	-0.001 (0.052)	9.84E-01	6.54E-01	-0.059 (0.054)	2.73E-01	2.35E-01
LDL cholesterol	0.002 (0.058)	9.70E-01	6.52E-01	0.024 (0.060)	6.90E-01	3.53E-01
TG	0.092 (0.080)	2.51E-01	4.23E-01	0.075 (0.083)	3.65E-01	2.66E-01
Phospholipids						
LysoPC a C14:0	-0.018 (0.039)	6.45E-01	5.89E-01	-0.080 (0.041)	5.04E-02	8.61E-02
LysoPC a C16:0	0.051 (0.046)	2.68E-01	4.23E-01	-0.012 (0.047)	8.07E-01	3.74E-01
LysoPC a C16:1	-0.019 (0.047)	6.84E-01	5.89E-01	-0.050 (0.049)	3.07E-01	2.46E-01
LysoPC a C17:0	-0.023 (0.043)	5.93E-01	5.81E-01	-0.056 (0.045)	2.16E-01	2.02E-01
LysoPC a C18:0	0.074 (0.054)	1.72E-01	3.99E-01	-0.012 (0.056)	8.36E-01	3.81E-01
LysoPC a C18:1	0.028 (0.044)	5.22E-01	5.46E-01	0.010 (0.045)	8.17E-01	3.75E-01
LysoPC a C18:2	-0.072 (0.059)	2.29E-01	4.13E-01	-0.108 (0.062)	8.03E-02	1.13E-01
LysoPC a C18:3	-0.070 (0.060)	2.45E-01	4.23E-01	-0.126 (0.062)	4.43E-02	8.01E-02
LysoPC a C20:3	-0.021 (0.057)	7.12E-01	5.89E-01	-0.056 (0.060)	3.51E-01	2.62E-01
LysoPC a C20:4	0.061 (0.057)	2.89E-01	4.35E-01	0.070 (0.060)	2.40E-01	2.15E-01
LysoPC a C20:5	-0.026 (0.079)	7.40E-01	5.89E-01	-0.142 (0.082)	8.53E-02	1.16E-01
LysoPC a C22:5	0.038 (0.076)	6.15E-01	5.81E-01	0.061 (0.079)	4.39E-01	2.87E-01
LysoPC a C22:6	0.029 (0.065)	6.58E-01	5.89E-01	-0.055 (0.068)	4.15E-01	2.84E-01
LysoPC a C24:0	0.001 (0.037)	9.71E-01	6.52E-01	0.016 (0.038)	6.72E-01	3.53E-01
LysoPC a C26:0	0.158 (0.062)	1.18E-02	1.96E-01	0.098 (0.065)	1.29E-01	1.48E-01
LysoPC a C26:1	0.047 (0.018)	8.30E-03	1.96E-01	0.049 (0.018)	8.79E-03	**3.09E-02**
LysoPC a C28:0	0.006 (0.028)	8.41E-01	6.15E-01	-0.012 (0.030)	6.81E-01	3.53E-01
LysoPC a C28:1	0.077 (0.056)	1.70E-01	3.99E-01	0.073 (0.058)	2.13E-01	2.02E-01
PC aa C24:0	0.130 (0.061)	3.49E-02	2.23E-01	0.180 (0.064)	5.02E-03	**2.40E-02**
PC aa C26:0	0.090 (0.037)	1.57E-02	2.23E-01	0.112 (0.038)	3.57E-03	**1.86E-02**

PC aa C28:1	-0.038 (0.048)	4.26E-01	5.02E-01	-0.052 (0.049)	2.98E-01	2.46E-01
PC aa C30:0	-0.079 (0.051)	1.20E-01	3.68E-01	-0.083 (0.053)	1.19E-01	1.42E-01
PC aa C32:0	-0.005 (0.055)	9.21E-01	6.42E-01	-0.008 (0.057)	8.91E-01	3.90E-01
PC aa C32:1	-0.118 (0.083)	1.58E-01	3.95E-01	-0.075 (0.087)	3.84E-01	2.70E-01
PC aa C32:3	-0.010 (0.048)	8.35E-01	6.15E-01	-0.053 (0.050)	2.93E-01	2.46E-01
PC aa C34:1	-0.005 (0.047)	9.21E-01	6.42E-01	-0.018 (0.049)	7.20E-01	3.61E-01
PC aa C34:2	-0.040 (0.059)	4.99E-01	5.40E-01	-0.056 (0.061)	3.55E-01	2.62E-01
PC aa C34:3	-0.057 (0.065)	3.87E-01	4.86E-01	-0.043 (0.068)	5.28E-01	3.13E-01
PC aa C34:4	0.016 (0.050)	7.43E-01	5.89E-01	0.029 (0.052)	5.70E-01	3.26E-01
PC aa C36:0	-0.031 (0.061)	6.05E-01	5.81E-01	-0.080 (0.063)	2.05E-01	1.96E-01
PC aa C36:1	-0.025 (0.045)	5.85E-01	5.81E-01	-0.055 (0.047)	2.43E-01	2.16E-01
PC aa C36:2	-0.046 (0.053)	3.92E-01	4.86E-01	-0.089 (0.055)	1.09E-01	1.36E-01
PC aa C36:3	-0.037 (0.052)	4.79E-01	5.32E-01	-0.081 (0.054)	1.37E-01	1.50E-01
PC aa C36:4	0.066 (0.043)	1.26E-01	3.70E-01	0.072 (0.045)	1.11E-01	1.36E-01
PC aa C36:5	-0.082 (0.068)	2.28E-01	4.13E-01	-0.144 (0.071)	4.27E-02	7.99E-02
PC aa C36:6	-0.004 (0.052)	9.45E-01	6.50E-01	-0.067 (0.054)	2.16E-01	2.02E-01
PC aa C38:0	-0.018 (0.042)	6.68E-01	5.89E-01	-0.029 (0.044)	5.06E-01	3.06E-01
PC aa C38:1	-0.010 (0.266)	9.70E-01	6.52E-01	0.372 (0.268)	1.67E-01	1.73E-01
PC aa C38:3	-0.022 (0.048)	6.51E-01	5.89E-01	-0.088 (0.050)	7.75E-02	1.12E-01
PC aa C38:4	0.090 (0.042)	3.48E-02	2.23E-01	0.083 (0.044)	6.19E-02	9.86E-02
PC aa C38:5	0.025 (0.041)	5.42E-01	5.58E-01	0.038 (0.043)	3.78E-01	2.69E-01
PC aa C38:6	0.028 (0.053)	5.96E-01	5.81E-01	-0.060 (0.055)	2.73E-01	2.35E-01
PC aa C40:1	-0.001 (0.039)	9.75E-01	6.52E-01	-0.040 (0.041)	3.24E-01	2.54E-01
PC aa C40:2	0.041 (0.060)	4.97E-01	5.40E-01	-0.027 (0.063)	6.72E-01	3.53E-01
PC aa C40:3	0.000 (0.041)	9.94E-01	6.58E-01	0.000 (0.042)	1.00E+00	4.22E-01
PC aa C40:4	0.042 (0.044)	3.35E-01	4.48E-01	0.043 (0.045)	3.41E-01	2.58E-01
PC aa C40:5	0.039 (0.062)	5.22E-01	5.46E-01	0.053 (0.064)	4.12E-01	2.83E-01
PC aa C40:6	0.036 (0.057)	5.31E-01	5.49E-01	-0.078 (0.060)	1.94E-01	1.91E-01
PC aa C42:0	-0.003 (0.051)	9.56E-01	6.51E-01	-0.040 (0.053)	4.46E-01	2.87E-01
PC aa C42:1	0.042 (0.047)	3.74E-01	4.73E-01	-0.004 (0.049)	9.28E-01	4.02E-01
PC aa C42:2	0.055 (0.041)	1.79E-01	3.99E-01	0.017 (0.043)	6.85E-01	3.53E-01
PC aa C42:4	0.047 (0.038)	2.18E-01	4.13E-01	0.056 (0.040)	1.59E-01	1.70E-01
PC aa C42:5	0.096 (0.045)	3.30E-02	2.23E-01	0.076 (0.047)	1.04E-01	1.33E-01
PC aa C42:6	0.063 (0.044)	1.49E-01	3.95E-01	0.044 (0.045)	3.32E-01	2.56E-01
PC ae C30:0	-0.097 (0.053)	6.73E-02	2.55E-01	-0.113 (0.055)	4.08E-02	7.86E-02
PC ae C30:1	1.927 (0.895)	3.69E-02	2.23E-01	1.815 (0.894)	4.84E-02	8.39E-02
PC ae C30:2	-0.015 (0.044)	7.33E-01	5.89E-01	-0.017 (0.045)	7.07E-01	3.57E-01
PC ae C32:1	-0.057 (0.058)	3.24E-01	4.44E-01	-0.035 (0.060)	5.59E-01	3.23E-01
PC ae C32:2	-0.059 (0.057)	3.00E-01	4.35E-01	-0.043 (0.059)	4.62E-01	2.92E-01
PC ae C34:0	-0.061 (0.059)	3.03E-01	4.35E-01	-0.086 (0.062)	1.62E-01	1.71E-01
PC ae C34:1	-0.052 (0.054)	3.29E-01	4.44E-01	-0.054 (0.056)	3.34E-01	2.56E-01
PC ae C34:2	-0.021 (0.051)	6.83E-01	5.89E-01	-0.010 (0.053)	8.47E-01	3.81E-01
PC ae C34:3	-0.023 (0.059)	6.99E-01	5.89E-01	-0.041 (0.062)	5.07E-01	3.06E-01
PC ae C36:0	0.016 (0.047)	7.31E-01	5.89E-01	-0.024 (0.049)	6.19E-01	3.43E-01
PC ae C36:1	-0.041 (0.048)	4.01E-01	4.91E-01	-0.058 (0.050)	2.48E-01	2.18E-01

PC ae C36:2	-0.055 (0.053)	3.01E-01	4.35E-01	-0.066 (0.055)	2.35E-01	2.14E-01
PC ae C36:3	-0.072 (0.054)	1.81E-01	3.99E-01	-0.090 (0.056)	1.08E-01	1.36E-01
PC ae C36:4	-0.003 (0.044)	9.48E-01	6.50E-01	-0.005 (0.046)	9.12E-01	3.96E-01
PC ae C36:5	0.027 (0.052)	6.03E-01	5.81E-01	0.045 (0.054)	3.99E-01	2.76E-01
PC ae C38:0	0.008 (0.048)	8.64E-01	6.25E-01	-0.033 (0.050)	5.06E-01	3.06E-01
PC ae C38:1	-0.080 (0.076)	2.93E-01	4.35E-01	-0.034 (0.079)	6.64E-01	3.53E-01
PC ae C38:2	-0.156 (0.080)	5.10E-02	2.39E-01	-0.236 (0.083)	4.51E-03	**2.24E-02**
PC ae C38:3	-0.069 (0.048)	1.55E-01	3.95E-01	-0.113 (0.050)	2.56E-02	6.50E-02
PC ae C38:4	0.011 (0.044)	8.09E-01	6.08E-01	0.014 (0.046)	7.55E-01	3.71E-01
PC ae C38:5	0.025 (0.046)	5.94E-01	5.81E-01	0.055 (0.048)	2.52E-01	2.20E-01
PC ae C38:6	-0.013 (0.049)	7.83E-01	6.07E-01	-0.024 (0.051)	6.36E-01	3.43E-01
PC ae C40:1	0.065 (0.050)	1.94E-01	3.99E-01	0.012 (0.052)	8.20E-01	3.75E-01
PC ae C40:2	-0.032 (0.046)	4.83E-01	5.32E-01	-0.049 (0.048)	3.05E-01	2.46E-01
PC ae C40:3	0.004 (0.043)	9.29E-01	6.42E-01	-0.040 (0.045)	3.73E-01	2.68E-01
PC ae C40:4	0.007 (0.047)	8.81E-01	6.32E-01	-0.002 (0.049)	9.60E-01	4.11E-01
PC ae C40:5	0.018 (0.043)	6.73E-01	5.89E-01	0.033 (0.044)	4.53E-01	2.90E-01
PC ae C40:6	-0.013 (0.049)	7.94E-01	6.08E-01	-0.061 (0.051)	2.29E-01	2.11E-01
PC ae C42:0	0.056 (0.037)	1.28E-01	3.70E-01	0.039 (0.038)	3.00E-01	2.46E-01
PC ae C42:1	0.061 (0.039)	1.18E-01	3.68E-01	0.067 (0.040)	9.63E-02	1.28E-01
PC ae C42:2	0.036 (0.036)	3.14E-01	4.35E-01	-0.014 (0.037)	6.98E-01	3.55E-01
PC ae C42:3	0.035 (0.043)	4.16E-01	4.99E-01	-0.012 (0.044)	7.86E-01	3.71E-01
PC ae C42:4	0.026 (0.049)	5.97E-01	5.81E-01	0.021 (0.051)	6.85E-01	3.53E-01
PC ae C42:5	0.015 (0.043)	7.22E-01	5.89E-01	-0.002 (0.044)	9.64E-01	4.12E-01
PC ae C44:3	0.076 (0.035)	2.98E-02	2.23E-01	0.056 (0.036)	1.23E-01	1.44E-01
PC ae C44:4	0.031 (0.043)	4.73E-01	5.32E-01	0.009 (0.045)	8.39E-01	3.81E-01
PC ae C44:5	0.049 (0.053)	3.47E-01	4.55E-01	0.026 (0.055)	6.33E-01	3.43E-01
PC ae C44:6	0.043 (0.052)	4.10E-01	4.95E-01	0.007 (0.054)	8.94E-01	3.90E-01
SM (OH) C14:1	-0.101 (0.051)	4.62E-02	2.39E-01	-0.146 (0.053)	5.97E-03	**2.46E-02**
SM (OH) C16:1	-0.092 (0.048)	5.71E-02	2.39E-01	-0.134 (0.050)	8.24E-03	**2.98E-02**
SM (OH) C22:1	-0.122 (0.054)	2.59E-02	2.23E-01	-0.134 (0.057)	1.85E-02	5.25E-02
SM (OH) C22:2	-0.104 (0.057)	7.19E-02	2.59E-01	-0.104 (0.060)	8.22E-02	1.13E-01
SM (OH) C24:1	-0.097 (0.051)	5.66E-02	2.39E-01	-0.106 (0.053)	4.55E-02	8.01E-02
SM C16:0	-0.056 (0.042)	1.90E-01	3.99E-01	-0.072 (0.044)	1.03E-01	1.33E-01
SM C16:1	-0.059 (0.049)	2.23E-01	4.13E-01	-0.104 (0.051)	3.97E-02	7.77E-02
SM C18:0	-0.036 (0.045)	4.21E-01	4.99E-01	-0.071 (0.047)	1.33E-01	1.50E-01
SM C18:1	-0.026 (0.052)	6.17E-01	5.81E-01	-0.097 (0.054)	7.44E-02	1.10E-01
SM C20:2	-0.031 (0.056)	5.78E-01	5.81E-01	-0.080 (0.058)	1.69E-01	1.73E-01
SM C22:3	0.197 (0.078)	1.16E-02	1.96E-01	0.195 (0.081)	1.61E-02	**4.90E-02**
SM C24:0	-0.108 (0.043)	1.26E-02	1.96E-01	-0.133 (0.045)	3.15E-03	**1.71E-02**
SM C24:1	-0.070 (0.047)	1.35E-01	3.83E-01	-0.078 (0.049)	1.11E-01	1.36E-01
SM C26:0	-0.102 (0.050)	4.36E-02	2.33E-01	-0.078 (0.052)	1.37E-01	1.50E-01
SM C26:1	-0.089 (0.046)	5.22E-02	2.39E-01	-0.104 (0.047)	2.90E-02	6.80E-02
Ratios	**β-estimate (SE)**	p-Value	**q-Value**	**β-estimate (SE)**	p-Value	**q-Value**
Acyl-carnitines						

(AC C16:0 + AC C18:0) / AC C0	0.005 (0.082)	9.52E-01	6.51E-01	-0.061 (0.085)	4.74E-01	2.97E-01
(AC C2:0 + AC C3:0) / AC C0	-0.027 (0.040)	5.05E-01	5.40E-01	0.008 (0.042)	8.53E-01	3.81E-01
AC C12:0 / AC C10:0	-0.011 (0.047)	8.07E-01	6.08E-01	-0.029 (0.049)	5.54E-01	3.21E-01
AC C14:0 / AC C16:1	-0.075 (0.035)	3.11E-02	2.23E-01	-0.194 (0.036)	1.55E-07	**9.27E-06**
AC C2:0 / AC C0	-0.032 (0.044)	4.71E-01	5.32E-01	-0.016 (0.046)	7.32E-01	3.65E-01
AC C3:0 / AC C4:0	-0.021 (0.082)	7.97E-01	6.08E-01	0.136 (0.085)	1.10E-01	1.36E-01
Amino acids						
Cit / Arg	0.137 (0.107)	2.00E-01	4.04E-01	0.380 (0.111)	6.70E-04	**5.73E-03**
Cit / Orn	-0.026 (0.039)	5.08E-01	5.40E-01	0.042 (0.041)	3.07E-01	2.46E-01
Fisher	0.032 (0.030)	2.92E-01	4.35E-01	0.053 (0.031)	9.32E-02	1.25E-01
Gly / PC ae C38:2	0.079 (0.081)	3.28E-01	4.44E-01	0.097 (0.084)	2.46E-01	2.18E-01
Orn / Arg	0.163 (0.109)	1.37E-01	3.83E-01	0.338 (0.114)	3.08E-03	**1.71E-02**
Orn / Ser	0.077 (0.042)	6.94E-02	2.55E-01	0.096 (0.044)	3.01E-02	6.93E-02
Tyr / Phe	0.027 (0.025)	2.85E-01	4.35E-01	0.027 (0.026)	3.00E-01	2.46E-01
Bile acids						
Conj. BA / unconj. BA	-0.119 (0.168)	4.79E-01	5.32E-01	-0.159 (0.174)	3.63E-01	2.66E-01
Prim. BA / sec. BA	-0.038 (0.168)	8.21E-01	6.15E-01	-0.120 (0.175)	4.91E-01	3.06E-01
Phospholipids						
LysoPC / PC	0.017 (0.042)	6.92E-01	5.89E-01	-0.016 (0.044)	7.09E-01	3.57E-01
MUFA PC / SFA PC	-0.002 (0.025)	9.29E-01	6.42E-01	-0.002 (0.026)	9.46E-01	4.08E-01
PC aa C36:3 / PC aa C36:4	-0.103 (0.048)	3.36E-02	2.23E-01	-0.153 (0.050)	2.59E-03	**1.55E-02**
PC aa C40:3 / PC aa C42:5	-0.096 (0.042)	2.13E-02	2.23E-01	-0.076 (0.043)	7.79E-02	1.12E-01
PC ae C32:1 / PC ae C34:1	-0.005 (0.027)	8.65E-01	6.25E-01	0.019 (0.028)	4.99E-01	3.06E-01
PC ae C38:1 / PC aa C28:1	-0.036 (0.069)	6.00E-01	5.81E-01	0.055 (0.072)	4.44E-01	2.87E-01
PC ae C44:5 / PC ae C42:5	0.034 (0.020)	8.80E-02	2.99E-01	0.028 (0.021)	1.79E-01	1.80E-01
PUFA-PC / MUFA-PC	0.029 (0.026)	2.79E-01	4.32E-01	0.016 (0.027)	5.50E-01	3.21E-01
PUFA-PC / SFA-PC	0.026 (0.022)	2.41E-01	4.23E-01	0.015 (0.023)	5.30E-01	3.13E-01
SM (OH) C24:1 / SM C16:0	-0.041 (0.036)	2.58E-01	4.23E-01	-0.033 (0.038)	3.75E-01	2.68E-01
Sums	**β-estimate (SE)**	p-Value	**q-Value**	**β-estimate (SE)**	p-Value	**q-Value**
Acyl-carnitines						
AC C16:0 + AC C18:0	0.107 (0.083)	1.99E-01	4.04E-01	0.067 (0.086)	4.36E-01	2.87E-01
AC C2:0 + AC C3:0	0.064 (0.058)	2.66E-01	4.23E-01	0.124 (0.060)	3.91E-02	7.77E-02
Carnitines	0.058 (0.052)	2.69E-01	4.23E-01	0.115 (0.055)	3.55E-02	7.52E-02
Even. carn	0.067 (0.056)	2.28E-01	4.13E-01	0.109 (0.058)	6.06E-02	9.80E-02
Long. carn	0.108 (0.081)	1.82E-01	3.99E-01	0.117 (0.084)	1.67E-01	1.73E-01
Medium. carn	0.064 (0.068)	3.44E-01	4.55E-01	0.076 (0.071)	2.82E-01	2.41E-01
Odd. carn	0.020 (0.053)	7.06E-01	5.89E-01	0.134 (0.055)	1.61E-02	**4.90E-02**
Amino acids						
AA	-0.023 (0.021)	2.79E-01	4.32E-01	-0.054 (0.022)	1.64E-02	**4.90E-02**
Arom. AA	0.022 (0.024)	3.55E-01	4.56E-01	0.019 (0.024)	4.42E-01	2.87E-01
BCAA	0.054 (0.033)	1.01E-01	3.22E-01	0.077 (0.034)	2.57E-02	6.50E-02
Ess. AA	0.009 (0.023)	6.79E-01	5.89E-01	-0.006 (0.024)	7.87E-01	3.71E-01
Glucog. AA	-0.046 (0.031)	1.40E-01	3.85E-01	-0.105 (0.033)	1.44E-03	**1.07E-02**

Noness. AA	-0.045 (0.025)	6.90E-02	2.55E-01	-0.089 (0.026)	6.39E-04	**5.73E-03**
Bile acids						
Conj. BA	-0.105 (0.116)	3.63E-01	4.63E-01	-0.032 (0.121)	7.91E-01	3.71E-01
Prim. BA	-0.105 (0.113)	3.53E-01	4.56E-01	-0.021 (0.118)	8.56E-01	3.81E-01
Sec. BA	-0.021 (0.156)	8.92E-01	6.37E-01	0.129 (0.163)	4.26E-01	2.87E-01
Unconj. BA	0.042 (0.114)	7.15E-01	5.89E-01	0.119 (0.119)	3.18E-01	2.53E-01
Phospholipids						
Long. PC	0.029 (0.045)	5.14E-01	5.44E-01	-0.036 (0.047)	4.40E-01	2.87E-01
Long. PC aa	0.034 (0.051)	5.02E-01	5.40E-01	-0.046 (0.053)	3.84E-01	2.70E-01
Long. PC ae	0.017 (0.039)	6.64E-01	5.89E-01	-0.007 (0.041)	8.57E-01	3.81E-01
Long. SM	-0.084 (0.044)	5.66E-02	2.39E-01	-0.096 (0.045)	3.57E-02	7.52E-02
Long. SM C	-0.083 (0.044)	5.82E-02	2.39E-01	-0.096 (0.046)	3.65E-02	7.52E-02
Long. SM OH	-0.097 (0.051)	5.66E-02	2.39E-01	-0.106 (0.053)	4.55E-02	8.01E-02
LysoPC	0.040 (0.042)	3.45E-01	4.55E-01	-0.013 (0.044)	7.74E-01	3.71E-01
Mono. PC	-0.015 (0.047)	7.51E-01	5.91E-01	-0.026 (0.049)	5.93E-01	3.35E-01
Mono. PC aa	-0.013 (0.048)	7.78E-01	6.07E-01	-0.025 (0.050)	6.13E-01	3.41E-01
Mono. PC ae	-0.039 (0.048)	4.18E-01	4.99E-01	-0.042 (0.050)	3.98E-01	2.76E-01
PC	0.008 (0.040)	8.33E-01	6.15E-01	-0.013 (0.041)	7.58E-01	3.71E-01
PC aa	0.010 (0.040)	8.04E-01	6.08E-01	-0.013 (0.041)	7.56E-01	3.71E-01
PC ae	-0.009 (0.043)	8.33E-01	6.15E-01	-0.012 (0.045)	7.88E-01	3.71E-01
Poly. PC	0.014 (0.039)	7.30E-01	5.89E-01	-0.010 (0.041)	8.10E-01	3.74E-01
Poly. PC aa	0.015 (0.039)	7.00E-01	5.89E-01	-0.010 (0.041)	8.02E-01	3.74E-01
Poly. PC ae	-0.005 (0.043)	9.11E-01	6.42E-01	-0.006 (0.045)	8.94E-01	3.90E-01
Sat. LysoPC	0.051 (0.046)	2.59E-01	4.23E-01	-0.013 (0.047)	7.81E-01	3.71E-01
Sat. PC	-0.013 (0.047)	7.84E-01	6.07E-01	-0.025 (0.049)	6.14E-01	3.41E-01
Sat. PC aa	-0.013 (0.049)	7.88E-01	6.07E-01	-0.020 (0.051)	6.92E-01	3.53E-01
Sat. PC ae	-0.009 (0.044)	8.43E-01	6.15E-01	-0.043 (0.046)	3.53E-01	2.62E-01
Saturmono. PC	-0.015 (0.047)	7.51E-01	5.91E-01	-0.026 (0.049)	5.93E-01	3.35E-01
Short. PC	-0.017 (0.050)	7.27E-01	5.89E-01	-0.025 (0.052)	6.26E-01	3.43E-01
Short. PC aa	-0.017 (0.050)	7.41E-01	5.89E-01	-0.025 (0.052)	6.32E-01	3.43E-01
Short. PC ae	-0.053 (0.052)	3.07E-01	4.35E-01	-0.052 (0.054)	3.33E-01	2.56E-01
SM	-0.060 (0.043)	1.64E-01	3.98E-01	-0.084 (0.045)	6.29E-02	9.89E-02
SM (OH)	-0.107 (0.051)	3.60E-02	2.23E-01	-0.123 (0.053)	2.04E-02	5.53E-02
SM C	-0.056 (0.043)	1.92E-01	3.99E-01	-0.081 (0.045)	7.23E-02	1.08E-01
Unsat. lysoPC	0.007 (0.045)	8.80E-01	6.32E-01	-0.013 (0.047)	7.76E-01	3.71E-01
Very. lysoPC	0.044 (0.021)	3.85E-02	2.25E-01	0.039 (0.022)	7.17E-02	1.08E-01

Data are β-estimate (SE) of UCB metabolite concentrations, sums and ratios in offspring of obese and offspring of severely obese mothers in reference to offspring of normal weight mothers. Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. GA, gestational age; GWG, gestational weight gain; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error; TG, triglyceride; UCB, umbilical cord blood.

Table S 6: Association of maternal preconception obesity and UCB metabolites stratified by offspring sex

	Male						Female					
	Offspring of obese vs. offspring of normal weight mothers			Offspring of severely obese vs. offspring of normal weight mothers			Offspring of obese vs. offspring of normal weight mothers			Offspring of severely obese vs. offspring of normal weight mothers		
Metabolite	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Acyl-carnitines												
AC 2-M-C3:0	0.242 (0.109)	2.72E-02	3.68E-01	0.351 (0.120)	3.89E-03	6.89E-02	0.113 (0.105)	2.80E-01	6.39E-01	0.237 (0.102)	2.14E-02	6.14E-02
AC 2-M-C3:1	0.215 (0.153)	1.64E-01	6.36E-01	0.327 (0.170)	5.61E-02	3.17E-01	-0.001 (0.143)	9.94E-01	8.93E-01	0.156 (0.140)	2.68E-01	2.47E-01
AC 2-M-C4:0	0.181 (0.116)	1.21E-01	5.60E-01	0.352 (0.128)	6.65E-03	7.91E-02	0.137 (0.112)	2.22E-01	6.38E-01	0.278 (0.109)	1.18E-02	**4.16E-02**
AC 3-M-C4:0	0.452 (0.188)	1.75E-02	2.87E-01	0.682 (0.208)	1.27E-03	**3.52E-02**	0.168 (0.207)	4.18E-01	7.06E-01	0.135 (0.203)	5.07E-01	3.22E-01
AC C0	0.114 (0.060)	6.09E-02	4.17E-01	0.167 (0.067)	1.30E-02	1.24E-01	0.082 (0.056)	1.43E-01	4.96E-01	0.077 (0.055)	1.59E-01	1.93E-01
AC C10:0	0.180 (0.102)	8.08E-02	4.65E-01	0.188 (0.113)	9.87E-02	4.06E-01	-0.037 (0.110)	7.37E-01	8.49E-01	0.047 (0.108)	6.66E-01	3.56E-01
AC C10:1	0.121 (0.094)	2.01E-01	6.43E-01	0.171 (0.103)	9.99E-02	4.06E-01	0.010 (0.091)	9.16E-01	8.76E-01	0.082 (0.089)	3.55E-01	2.70E-01
AC C11:0	0.253 (0.131)	5.52E-02	4.17E-01	-0.001 (0.144)	9.94E-01	7.76E-01	-0.076 (0.107)	4.78E-01	7.47E-01	0.025 (0.105)	8.12E-01	3.86E-01
AC C12:0	0.134 (0.114)	2.42E-01	6.55E-01	0.092 (0.126)	4.66E-01	6.58E-01	-0.090 (0.107)	4.01E-01	6.96E-01	0.033 (0.104)	7.54E-01	3.69E-01
AC C12:1	0.179 (0.113)	1.17E-01	5.60E-01	0.176 (0.125)	1.59E-01	4.89E-01	-0.091 (0.109)	4.05E-01	6.96E-01	0.028 (0.107)	7.94E-01	3.84E-01
AC C13:0	0.108 (0.117)	3.59E-01	6.55E-01	-0.065 (0.130)	6.16E-01	7.07E-01	-0.173 (0.114)	1.31E-01	4.67E-01	-0.053 (0.112)	6.36E-01	3.47E-01
AC C14:0	0.033 (0.134)	8.05E-01	7.05E-01	-0.036 (0.148)	8.11E-01	7.42E-01	-0.039 (0.119)	7.42E-01	8.49E-01	0.007 (0.117)	9.50E-01	4.22E-01
AC C14:1	0.184 (0.129)	1.57E-01	6.36E-01	0.156 (0.143)	2.77E-01	5.78E-01	-0.020 (0.116)	8.67E-01	8.76E-01	0.112 (0.114)	3.29E-01	2.63E-01
AC C15:0	0.060 (0.144)	6.75E-01	6.67E-01	-0.106 (0.159)	5.06E-01	6.68E-01	-0.081 (0.126)	5.20E-01	7.83E-01	-0.039 (0.123)	7.50E-01	3.69E-01
AC C16:0	0.104 (0.129)	4.23E-01	6.55E-01	0.021 (0.142)	8.82E-01	7.57E-01	0.036 (0.119)	7.59E-01	8.49E-01	0.062 (0.116)	5.97E-01	3.35E-01
AC C16:1	0.158 (0.138)	2.54E-01	6.55E-01	0.197 (0.152)	1.99E-01	5.15E-01	-0.011 (0.128)	9.34E-01	8.76E-01	0.156 (0.125)	2.14E-01	2.14E-01
AC C17:0	0.050 (0.124)	6.88E-01	6.67E-01	-0.054 (0.137)	6.95E-01	7.07E-01	-0.052 (0.108)	6.31E-01	8.19E-01	-0.057 (0.106)	5.90E-01	3.35E-01
AC C18:0	0.091 (0.111)	4.13E-01	6.55E-01	-0.006 (0.123)	9.60E-01	7.73E-01	-0.032 (0.097)	7.40E-01	8.49E-01	0.043 (0.095)	6.50E-01	3.51E-01
AC C18:1	0.117 (0.125)	3.51E-01	6.55E-01	0.144 (0.138)	2.97E-01	5.78E-01	0.037 (0.112)	7.40E-01	8.49E-01	0.192 (0.110)	8.18E-02	1.35E-01
AC C18:2	0.015 (0.140)	9.16E-01	7.38E-01	0.118 (0.155)	4.47E-01	6.52E-01	0.072 (0.126)	5.69E-01	7.95E-01	0.126 (0.124)	3.12E-01	2.60E-01
AC C2:0	0.101 (0.091)	2.67E-01	6.55E-01	0.143 (0.100)	1.57E-01	4.89E-01	-0.007 (0.083)	9.36E-01	8.76E-01	0.033 (0.081)	6.88E-01	3.58E-01
AC C3:0	0.244 (0.110)	2.78E-02	3.68E-01	0.429 (0.122)	5.31E-04	**1.99E-02**	-0.007 (0.096)	9.42E-01	8.77E-01	0.174 (0.094)	6.53E-02	1.22E-01

AC C3-DC	0.051 (0.075)	4.92E-01	6.55E-01	0.077 (0.083)	3.54E-01	6.07E-01	-0.038 (0.071)	5.91E-01	7.99E-01	0.026 (0.069)	7.05E-01	3.59E-01
AC C3-M-DC	0.008 (0.080)	9.17E-01	7.38E-01	-0.034 (0.088)	7.01E-01	7.07E-01	-0.147 (0.072)	4.27E-02	4.36E-01	-0.075 (0.070)	2.86E-01	2.52E-01
AC C4:0	0.113 (0.113)	3.18E-01	6.55E-01	0.247 (0.125)	5.07E-02	3.05E-01	0.043 (0.100)	6.67E-01	8.29E-01	-0.012 (0.098)	9.02E-01	4.09E-01
AC C4:1	0.179 (0.259)	4.92E-01	6.55E-01	0.404 (0.288)	1.63E-01	4.89E-01	0.121 (0.237)	6.10E-01	8.08E-01	0.382 (0.233)	1.03E-01	1.51E-01
AC C4-DC	-0.034 (0.115)	7.65E-01	6.95E-01	-0.127 (0.129)	3.26E-01	5.78E-01	-0.031 (0.100)	7.57E-01	8.49E-01	0.041 (0.098)	6.81E-01	3.56E-01
AC C4-OHa	0.110 (0.157)	4.84E-01	6.55E-01	0.202 (0.173)	2.45E-01	5.59E-01	-0.057 (0.152)	7.05E-01	8.37E-01	0.080 (0.149)	5.93E-01	3.35E-01
AC C4-OHb	0.085 (0.231)	7.12E-01	6.81E-01	0.342 (0.255)	1.82E-01	4.96E-01	-0.009 (0.215)	9.67E-01	8.85E-01	0.299 (0.211)	1.58E-01	1.93E-01
AC C5:1	0.245 (0.124)	4.89E-02	4.17E-01	0.383 (0.137)	5.73E-03	7.91E-02	0.073 (0.103)	4.79E-01	7.47E-01	0.140 (0.101)	1.68E-01	1.93E-01
AC C5-DC	0.064 (0.091)	4.86E-01	6.55E-01	-0.046 (0.101)	6.49E-01	7.07E-01	0.056 (0.088)	5.26E-01	7.87E-01	0.177 (0.085)	3.98E-02	9.60E-02
AC C5-M-DC	-0.948 (0.208)	1.01E-05	**2.15E-03**	-0.916 (0.230)	1.02E-04	**7.49E-03**	-0.509 (0.215)	1.88E-02	4.36E-01	-0.371 (0.210)	7.97E-02	1.35E-01
AC C5-OH	0.164 (0.100)	1.03E-01	5.49E-01	0.388 (0.110)	5.42E-04	**1.99E-02**	0.074 (0.084)	3.80E-01	6.96E-01	0.135 (0.082)	1.01E-01	1.51E-01
AC C6:0	0.349 (0.144)	1.65E-02	2.87E-01	0.438 (0.160)	6.66E-03	7.91E-02	0.093 (0.140)	5.08E-01	7.74E-01	0.106 (0.137)	4.41E-01	3.01E-01
AC C6:1	0.068 (0.124)	5.82E-01	6.55E-01	0.100 (0.136)	4.65E-01	6.58E-01	-0.032 (0.124)	7.94E-01	8.52E-01	-0.058 (0.121)	6.34E-01	3.47E-01
AC C6-DC	0.153 (0.180)	3.99E-01	6.55E-01	0.159 (0.199)	4.25E-01	6.52E-01	0.026 (0.181)	8.85E-01	8.76E-01	0.429 (0.178)	1.67E-02	5.42E-02
AC C6-OHa	0.234 (0.132)	7.77E-02	4.65E-01	0.425 (0.146)	4.04E-03	6.89E-02	0.058 (0.134)	6.66E-01	8.29E-01	0.185 (0.131)	1.62E-01	1.93E-01
AC C6-OHb	0.166 (0.114)	1.44E-01	6.28E-01	0.281 (0.125)	2.63E-02	2.15E-01	0.046 (0.112)	6.83E-01	8.29E-01	0.111 (0.110)	3.14E-01	2.60E-01
AC C7:0	0.255 (0.106)	1.74E-02	2.87E-01	0.099 (0.117)	3.97E-01	6.43E-01	0.021 (0.107)	8.47E-01	8.76E-01	0.024 (0.105)	8.22E-01	3.89E-01
AC C7-DC	-0.102 (0.105)	3.31E-01	6.55E-01	-0.189 (0.116)	1.03E-01	4.06E-01	-0.159 (0.111)	1.56E-01	5.17E-01	0.047 (0.109)	6.67E-01	3.56E-01
AC C8:0	0.190 (0.104)	7.08E-02	4.44E-01	0.204 (0.115)	7.87E-02	3.65E-01	0.035 (0.116)	7.65E-01	8.49E-01	0.100 (0.113)	3.80E-01	2.77E-01
AC C8:1	0.286 (0.157)	6.98E-02	4.44E-01	0.504 (0.173)	4.06E-03	6.89E-02	0.166 (0.149)	2.67E-01	6.39E-01	0.274 (0.146)	6.12E-02	1.22E-01
AC C8-OH	0.153 (0.094)	1.06E-01	5.49E-01	0.106 (0.104)	3.07E-01	5.78E-01	-0.106 (0.106)	3.15E-01	6.39E-01	-0.027 (0.103)	7.93E-01	3.84E-01
AC C9:0	0.062 (0.133)	6.40E-01	6.56E-01	-0.141 (0.146)	3.37E-01	5.90E-01	-0.202 (0.143)	1.57E-01	5.17E-01	-0.253 (0.140)	7.19E-02	1.26E-01
AC iso-C11:0	0.155 (0.129)	2.30E-01	6.55E-01	0.196 (0.142)	1.69E-01	4.89E-01	-0.074 (0.112)	5.11E-01	7.74E-01	-0.092 (0.109)	4.01E-01	2.87E-01
AC iso-C13:0	-0.120 (0.141)	3.96E-01	6.55E-01	-0.254 (0.156)	1.05E-01	4.06E-01	-0.368 (0.150)	1.50E-02	4.36E-01	-0.386 (0.147)	9.18E-03	**3.53E-02**
AC iso-C15:0	0.008 (0.126)	9.47E-01	7.41E-01	-0.072 (0.140)	6.05E-01	7.07E-01	-0.084 (0.106)	4.32E-01	7.23E-01	-0.142 (0.104)	1.73E-01	1.94E-01
AC iso-C17:0	0.045 (0.127)	7.22E-01	6.84E-01	-0.046 (0.140)	7.45E-01	7.08E-01	-0.062 (0.117)	5.98E-01	8.01E-01	-0.070 (0.114)	5.40E-01	3.33E-01
AC iso-C9:0	0.123 (0.135)	3.61E-01	6.55E-01	0.005 (0.149)	9.76E-01	7.76E-01	-0.156 (0.143)	2.75E-01	6.39E-01	-0.136 (0.140)	3.33E-01	2.63E-01
Amino acids												
1-M-His	0.039 (0.086)	6.48E-01	6.57E-01	0.055 (0.095)	5.67E-01	7.00E-01	-0.147 (0.082)	7.50E-02	4.67E-01	0.106 (0.080)	1.91E-01	1.97E-01

3-M-His	0.502 (0.377)	1.85E-01	6.43E-01	0.580 (0.418)	1.68E-01	4.89E-01	-0.077 (0.367)	8.34E-01	8.76E-01	0.376 (0.360)	2.97E-01	2.56E-01
AAB	0.105 (0.107)	3.29E-01	6.55E-01	0.228 (0.119)	5.57E-02	3.17E-01	-0.102 (0.096)	2.91E-01	6.39E-01	-0.049 (0.094)	5.99E-01	3.35E-01
AADP	0.158 (0.100)	1.17E-01	5.60E-01	0.103 (0.111)	3.52E-01	6.07E-01	-0.013 (0.083)	8.77E-01	8.76E-01	0.057 (0.081)	4.79E-01	3.14E-01
Ala	-0.035 (0.057)	5.44E-01	6.55E-01	-0.094 (0.063)	1.37E-01	4.60E-01	0.003 (0.064)	9.63E-01	8.85E-01	-0.033 (0.063)	6.01E-01	3.35E-01
Anserine	0.078 (0.321)	8.08E-01	7.05E-01	-0.167 (0.361)	6.43E-01	7.07E-01	-0.180 (0.347)	6.04E-01	8.04E-01	0.220 (0.334)	5.12E-01	3.22E-01
Arg	0.070 (0.143)	6.26E-01	6.55E-01	-0.143 (0.157)	3.66E-01	6.21E-01	-0.344 (0.140)	1.50E-02	4.36E-01	-0.573 (0.137)	4.57E-05	**3.99E-03**
Asn	-0.043 (0.060)	4.73E-01	6.55E-01	-0.102 (0.066)	1.22E-01	4.35E-01	-0.163 (0.053)	2.55E-03	2.45E-01	-0.168 (0.052)	1.57E-03	**1.95E-02**
Asp	-0.082 (0.078)	2.96E-01	6.55E-01	-0.183 (0.086)	3.51E-02	2.42E-01	-0.193 (0.074)	9.52E-03	4.36E-01	-0.154 (0.072)	3.43E-02	8.92E-02
BAIB	-0.202 (0.183)	2.70E-01	6.55E-01	-0.052 (0.202)	7.98E-01	7.34E-01	-0.112 (0.159)	4.83E-01	7.47E-01	-0.087 (0.155)	5.75E-01	3.35E-01
Carnosine	0.054 (0.287)	8.51E-01	7.17E-01	-0.500 (0.318)	1.18E-01	4.32E-01	-0.192 (0.283)	4.98E-01	7.63E-01	-0.275 (0.272)	3.14E-01	2.60E-01
Cit	-0.065 (0.060)	2.82E-01	6.55E-01	0.011 (0.066)	8.71E-01	7.57E-01	-0.033 (0.061)	5.87E-01	7.99E-01	-0.034 (0.060)	5.72E-01	3.35E-01
Cys-Cys	-0.085 (0.086)	3.25E-01	6.55E-01	0.048 (0.095)	6.14E-01	7.07E-01	-0.155 (0.085)	7.09E-02	4.67E-01	0.044 (0.084)	5.98E-01	3.35E-01
Dimethylglycine	0.233 (0.095)	1.49E-02	2.87E-01	0.264 (0.105)	1.27E-02	1.24E-01	-0.187 (0.089)	3.79E-02	4.36E-01	-0.074 (0.088)	4.03E-01	2.87E-01
GABA	-0.006 (0.156)	9.71E-01	7.41E-01	-0.223 (0.172)	1.96E-01	5.15E-01	-0.212 (0.139)	1.30E-01	4.67E-01	-0.111 (0.136)	4.14E-01	2.90E-01
Gln	0.044 (0.066)	5.10E-01	6.55E-01	0.087 (0.073)	2.36E-01	5.59E-01	-0.088 (0.058)	1.30E-01	4.67E-01	-0.099 (0.057)	8.13E-02	1.35E-01
Glu	-0.071 (0.061)	2.45E-01	6.55E-01	-0.144 (0.068)	3.45E-02	2.42E-01	-0.071 (0.065)	2.75E-01	6.39E-01	-0.066 (0.063)	2.98E-01	2.56E-01
Gly	-0.073 (0.047)	1.23E-01	5.60E-01	-0.172 (0.052)	1.28E-03	**3.52E-02**	-0.075 (0.046)	1.02E-01	4.67E-01	-0.112 (0.045)	1.34E-02	**4.46E-02**
Hcys	0.018 (0.148)	9.06E-01	7.38E-01	-0.099 (0.164)	5.49E-01	6.96E-01	0.131 (0.153)	3.94E-01	6.96E-01	0.335 (0.151)	2.73E-02	7.46E-02
His	-0.110 (0.055)	4.57E-02	4.17E-01	-0.276 (0.061)	9.83E-06	**2.17E-03**	-0.080 (0.053)	1.31E-01	4.67E-01	-0.200 (0.052)	1.48E-04	**4.54E-03**
Ile	0.139 (0.066)	3.60E-02	3.83E-01	0.185 (0.073)	1.20E-02	1.24E-01	-0.064 (0.058)	2.77E-01	6.39E-01	0.018 (0.057)	7.52E-01	3.69E-01
Leu	0.136 (0.070)	5.23E-02	4.17E-01	0.136 (0.077)	7.89E-02	3.65E-01	-0.060 (0.058)	3.06E-01	6.39E-01	-0.033 (0.057)	5.64E-01	3.35E-01
Lys	0.051 (0.048)	2.89E-01	6.55E-01	0.024 (0.053)	6.58E-01	7.07E-01	-0.030 (0.040)	4.51E-01	7.32E-01	-0.013 (0.039)	7.47E-01	3.69E-01
Met	0.002 (0.042)	9.67E-01	7.41E-01	-0.030 (0.046)	5.18E-01	6.68E-01	-0.092 (0.041)	2.78E-02	4.36E-01	-0.122 (0.040)	2.93E-03	**2.41E-02**
OH-Pro	0.158 (0.048)	1.27E-03	9.04E-02	0.124 (0.053)	2.08E-02	1.77E-01	0.069 (0.045)	1.30E-01	4.67E-01	0.139 (0.044)	1.90E-03	**1.95E-02**
Orn	-0.040 (0.050)	4.27E-01	6.55E-01	-0.062 (0.055)	2.61E-01	5.59E-01	-0.018 (0.049)	7.08E-01	8.37E-01	-0.064 (0.048)	1.85E-01	1.97E-01
Phe	0.050 (0.040)	2.14E-01	6.43E-01	0.024 (0.044)	5.84E-01	7.07E-01	-0.010 (0.037)	7.85E-01	8.52E-01	-0.021 (0.036)	5.66E-01	3.35E-01
Pro	0.012 (0.038)	7.48E-01	6.90E-01	-0.048 (0.043)	2.57E-01	5.59E-01	-0.048 (0.038)	2.08E-01	6.17E-01	-0.020 (0.037)	5.97E-01	3.35E-01
Sarcosine	0.125 (0.082)	1.27E-01	5.66E-01	0.061 (0.090)	4.99E-01	6.68E-01	-0.047 (0.079)	5.51E-01	7.92E-01	0.013 (0.077)	8.71E-01	3.99E-01
Ser	-0.095 (0.056)	9.37E-02	5.12E-01	-0.162 (0.062)	9.67E-03	1.07E-01	-0.130 (0.058)	2.65E-02	4.36E-01	-0.180 (0.057)	1.80E-03	**1.95E-02**

Thr	-0.002 (0.054)	9.66E-01	7.41E-01	-0.005 (0.060)	9.29E-01	7.70E-01	-0.034 (0.062)	5.81E-01	7.95E-01	-0.063 (0.061)	3.01E-01	2.56E-01
Trp	0.085 (0.048)	7.85E-02	4.65E-01	0.100 (0.053)	6.19E-02	3.25E-01	-0.049 (0.050)	3.25E-01	6.53E-01	0.009 (0.049)	8.55E-01	3.96E-01
Tyr	0.101 (0.042)	1.72E-02	2.87E-01	0.064 (0.046)	1.71E-01	4.89E-01	-0.012 (0.043)	7.78E-01	8.52E-01	0.001 (0.042)	9.83E-01	4.30E-01
Val	0.131 (0.046)	4.93E-03	1.82E-01	0.156 (0.051)	2.53E-03	6.20E-02	0.014 (0.041)	7.28E-01	8.49E-01	0.039 (0.041)	3.32E-01	2.63E-01
β-Ala	-0.008 (0.115)	9.48E-01	7.41E-01	-0.074 (0.128)	5.62E-01	7.00E-01	0.023 (0.133)	8.63E-01	8.76E-01	0.131 (0.131)	3.21E-01	2.60E-01
Amino acid derivatives												
Betaine	0.207 (0.073)	5.13E-03	1.82E-01	0.299 (0.081)	2.97E-04	**1.64E-02**	-0.009 (0.060)	8.86E-01	8.76E-01	-0.011 (0.059)	8.52E-01	3.96E-01
Choline	0.036 (0.056)	5.18E-01	6.55E-01	0.000 (0.062)	9.96E-01	7.76E-01	-0.006 (0.064)	9.23E-01	8.76E-01	-0.024 (0.063)	7.04E-01	3.59E-01
Bile acids												
CA	-0.203 (0.197)	3.06E-01	6.55E-01	0.166 (0.218)	4.47E-01	6.52E-01	-0.117 (0.195)	5.48E-01	7.92E-01	-0.131 (0.191)	4.93E-01	3.18E-01
CDCA	0.065 (0.113)	5.65E-01	6.55E-01	0.235 (0.125)	6.13E-02	3.25E-01	0.126 (0.120)	2.96E-01	6.39E-01	0.130 (0.119)	2.75E-01	2.49E-01
DCA	0.090 (0.308)	7.70E-01	6.95E-01	0.228 (0.339)	5.02E-01	6.68E-01	0.187 (0.336)	5.79E-01	7.95E-01	0.363 (0.329)	2.71E-01	2.47E-01
GCA	0.065 (0.200)	7.48E-01	6.90E-01	-0.270 (0.222)	2.25E-01	5.51E-01	-0.407 (0.218)	6.30E-02	4.67E-01	-0.359 (0.213)	9.37E-02	1.44E-01
GCDCA	0.188 (0.204)	3.58E-01	6.55E-01	0.154 (0.225)	4.95E-01	6.68E-01	-0.342 (0.207)	1.01E-01	4.67E-01	-0.085 (0.203)	6.76E-01	3.56E-01
GDCA	-0.189 (0.295)	5.22E-01	6.55E-01	-0.156 (0.325)	6.32E-01	7.07E-01	-0.332 (0.291)	2.55E-01	6.39E-01	-0.222 (0.285)	4.38E-01	3.01E-01
GLCA	-0.303 (0.446)	4.99E-01	6.55E-01	-0.267 (0.510)	6.03E-01	7.07E-01	-0.862 (0.437)	5.18E-02	4.36E-01	-1.036 (0.430)	1.80E-02	5.63E-02
GUDCA	0.556 (0.290)	5.73E-02	4.17E-01	0.750 (0.321)	2.07E-02	1.77E-01	-0.596 (0.264)	2.52E-02	4.36E-01	-0.291 (0.257)	2.61E-01	2.45E-01
LCA	0.235 (0.424)	5.80E-01	6.55E-01	0.335 (0.435)	4.44E-01	6.52E-01	0.069 (0.458)	8.80E-01	8.76E-01	0.328 (0.472)	4.89E-01	3.17E-01
TCA	0.062 (0.217)	7.76E-01	6.95E-01	0.085 (0.240)	7.23E-01	7.07E-01	-0.244 (0.208)	2.42E-01	6.39E-01	-0.187 (0.203)	3.59E-01	2.70E-01
TCDCA	0.114 (0.184)	5.36E-01	6.55E-01	0.143 (0.203)	4.83E-01	6.68E-01	-0.152 (0.181)	4.02E-01	6.96E-01	0.036 (0.178)	8.41E-01	3.94E-01
TDCA	-0.097 (0.338)	7.74E-01	6.95E-01	0.050 (0.363)	8.90E-01	7.57E-01	-0.312 (0.307)	3.09E-01	6.39E-01	-0.175 (0.298)	5.58E-01	3.35E-01
TLCA	-0.158 (0.313)	6.15E-01	6.55E-01	0.005 (0.331)	9.89E-01	7.76E-01	-0.415 (0.289)	1.53E-01	5.14E-01	-0.314 (0.281)	2.66E-01	2.47E-01
TLCA-S	-0.601 (0.179)	9.65E-04	9.04E-02	-0.408 (0.198)	4.06E-02	2.63E-01	-0.303 (0.180)	9.34E-02	4.67E-01	-0.237 (0.176)	1.79E-01	1.97E-01
TUDCA	-0.147 (0.182)	4.20E-01	6.55E-01	0.140 (0.201)	4.86E-01	6.68E-01	-0.203 (0.176)	2.49E-01	6.39E-01	-0.088 (0.171)	6.06E-01	3.35E-01
UDCA	0.292 (0.322)	3.66E-01	6.55E-01	0.228 (0.339)	5.03E-01	6.68E-01	-0.290 (0.341)	3.98E-01	6.96E-01	-0.215 (0.328)	5.13E-01	3.22E-01
Lipids												
HDL cholesterol	0.024 (0.078)	7.60E-01	6.95E-01	-0.009 (0.087)	9.15E-01	7.65E-01	-0.036 (0.073)	6.27E-01	8.17E-01	-0.112 (0.072)	1.19E-01	1.64E-01
LDL cholesterol	0.092 (0.087)	2.91E-01	6.55E-01	0.143 (0.096)	1.37E-01	4.60E-01	-0.083 (0.082)	3.11E-01	6.39E-01	-0.096 (0.080)	2.34E-01	2.24E-01
TG	0.118 (0.118)	3.20E-01	6.55E-01	0.036 (0.131)	7.86E-01	7.31E-01	0.044 (0.114)	6.99E-01	8.33E-01	0.059 (0.111)	5.99E-01	3.35E-01

Phospholipids												
LysoPC a C14:0	-0.048 (0.060)	4.19E-01	6.55E-01	-0.117 (0.067)	8.06E-02	3.65E-01	0.002 (0.055)	9.77E-01	8.86E-01	-0.050 (0.054)	3.58E-01	2.70E-01
LysoPC a C16:0	0.035 (0.065)	5.95E-01	6.55E-01	-0.033 (0.072)	6.45E-01	7.07E-01	0.053 (0.069)	4.40E-01	7.27E-01	0.000 (0.067)	9.94E-01	4.33E-01
LysoPC a C16:1	-0.004 (0.072)	9.60E-01	7.41E-01	-0.061 (0.080)	4.46E-01	6.52E-01	-0.030 (0.065)	6.49E-01	8.28E-01	-0.034 (0.064)	5.98E-01	3.35E-01
LysoPC a C17:0	0.002 (0.068)	9.73E-01	7.41E-01	-0.055 (0.075)	4.64E-01	6.58E-01	-0.051 (0.058)	3.79E-01	6.96E-01	-0.059 (0.057)	3.01E-01	2.56E-01
LysoPC a C18:0	0.071 (0.077)	3.53E-01	6.55E-01	-0.019 (0.085)	8.25E-01	7.43E-01	0.072 (0.082)	3.80E-01	6.96E-01	-0.007 (0.081)	9.29E-01	4.19E-01
LysoPC a C18:1	0.031 (0.067)	6.40E-01	6.56E-01	-0.006 (0.074)	9.40E-01	7.71E-01	0.025 (0.061)	6.78E-01	8.29E-01	0.020 (0.060)	7.41E-01	3.69E-01
LysoPC a C18:2	-0.066 (0.091)	4.70E-01	6.55E-01	-0.082 (0.101)	4.19E-01	6.52E-01	-0.101 (0.083)	2.29E-01	6.39E-01	-0.133 (0.082)	1.04E-01	1.51E-01
LysoPC a C18:3	-0.095 (0.088)	2.82E-01	6.55E-01	-0.195 (0.098)	4.69E-02	2.95E-01	-0.093 (0.087)	2.85E-01	6.39E-01	-0.093 (0.085)	2.79E-01	2.51E-01
LysoPC a C20:3	-0.041 (0.084)	6.30E-01	6.55E-01	-0.093 (0.093)	3.21E-01	5.78E-01	0.012 (0.085)	8.86E-01	8.76E-01	-0.020 (0.083)	8.06E-01	3.86E-01
LysoPC a C20:4	0.088 (0.089)	3.22E-01	6.55E-01	0.110 (0.098)	2.61E-01	5.59E-01	0.008 (0.079)	9.19E-01	8.76E-01	0.031 (0.078)	6.94E-01	3.59E-01
LysoPC a C20:5	-0.048 (0.115)	6.77E-01	6.67E-01	-0.193 (0.127)	1.32E-01	4.60E-01	-0.072 (0.116)	5.38E-01	7.88E-01	-0.182 (0.114)	1.11E-01	1.57E-01
LysoPC a C22:5	-0.009 (0.110)	9.33E-01	7.39E-01	0.051 (0.121)	6.74E-01	7.07E-01	0.114 (0.111)	3.05E-01	6.39E-01	0.086 (0.108)	4.29E-01	2.97E-01
LysoPC a C22:6	0.058 (0.096)	5.46E-01	6.55E-01	-0.081 (0.106)	4.44E-01	6.52E-01	-0.025 (0.095)	7.97E-01	8.52E-01	-0.077 (0.093)	4.11E-01	2.89E-01
LysoPC a C24:0	-0.028 (0.055)	6.09E-01	6.55E-01	-0.064 (0.061)	2.92E-01	5.78E-01	0.002 (0.053)	9.77E-01	8.86E-01	0.056 (0.052)	2.86E-01	2.52E-01
LysoPC a C26:0	0.071 (0.090)	4.28E-01	6.55E-01	0.015 (0.099)	8.80E-01	7.57E-01	0.161 (0.090)	7.41E-02	4.67E-01	0.149 (0.088)	8.98E-02	1.40E-01
LysoPC a C26:1	0.031 (0.026)	2.32E-01	6.55E-01	0.062 (0.029)	3.27E-02	2.42E-01	0.053 (0.025)	3.80E-02	4.36E-01	0.038 (0.025)	1.31E-01	1.75E-01
LysoPC a C28:0	0.031 (0.042)	4.68E-01	6.55E-01	0.031 (0.047)	5.11E-01	6.68E-01	-0.003 (0.041)	9.35E-01	8.76E-01	-0.038 (0.040)	3.48E-01	2.69E-01
LysoPC a C28:1	0.198 (0.087)	2.45E-02	3.68E-01	0.211 (0.096)	2.96E-02	2.33E-01	-0.063 (0.074)	3.94E-01	6.96E-01	-0.063 (0.072)	3.89E-01	2.81E-01
PC aa C24:0	0.120 (0.087)	1.72E-01	6.42E-01	0.167 (0.097)	8.52E-02	3.65E-01	0.055 (0.090)	5.42E-01	7.88E-01	0.123 (0.089)	1.67E-01	1.93E-01
PC aa C26:0	0.086 (0.055)	1.16E-01	5.60E-01	0.054 (0.060)	3.69E-01	6.22E-01	0.056 (0.053)	2.85E-01	6.39E-01	0.132 (0.051)	1.10E-02	**3.98E-02**
PC aa C28:1	-0.005 (0.074)	9.52E-01	7.41E-01	0.025 (0.082)	7.61E-01	7.17E-01	-0.075 (0.064)	2.43E-01	6.39E-01	-0.146 (0.063)	2.17E-02	6.14E-02
PC aa C30:0	0.018 (0.076)	8.12E-01	7.05E-01	0.039 (0.084)	6.48E-01	7.07E-01	-0.160 (0.072)	2.75E-02	4.36E-01	-0.192 (0.070)	6.91E-03	**2.93E-02**
PC aa C32:0	0.096 (0.081)	2.38E-01	6.55E-01	0.107 (0.089)	2.32E-01	5.56E-01	-0.099 (0.079)	2.10E-01	6.17E-01	-0.112 (0.077)	1.47E-01	1.89E-01
PC aa C32:1	0.028 (0.120)	8.17E-01	7.05E-01	0.055 (0.133)	6.81E-01	7.07E-01	-0.241 (0.122)	4.98E-02	4.36E-01	-0.188 (0.120)	1.17E-01	1.63E-01
PC aa C32:3	0.055 (0.075)	4.65E-01	6.55E-01	0.009 (0.083)	9.13E-01	7.65E-01	-0.086 (0.065)	1.88E-01	5.73E-01	-0.120 (0.064)	6.18E-02	1.22E-01
PC aa C34:1	0.042 (0.071)	5.56E-01	6.55E-01	0.002 (0.078)	9.77E-01	7.76E-01	-0.049 (0.068)	4.74E-01	7.47E-01	-0.051 (0.066)	4.42E-01	3.01E-01
PC aa C34:2	0.036 (0.087)	6.83E-01	6.67E-01	0.048 (0.096)	6.18E-01	7.07E-01	-0.127 (0.083)	1.30E-01	4.67E-01	-0.154 (0.082)	6.16E-02	1.22E-01
PC aa C34:3	0.023 (0.097)	8.17E-01	7.05E-01	0.046 (0.108)	6.72E-01	7.07E-01	-0.139 (0.092)	1.34E-01	4.72E-01	-0.120 (0.091)	1.89E-01	1.97E-01

PC aa C34:4	0.070 (0.076)	3.59E-01	6.55E-01	0.102 (0.084)	2.27E-01	5.51E-01	-0.042 (0.068)	5.37E-01	7.88E-01	-0.037 (0.067)	5.81E-01	3.35E-01
PC aa C36:0	0.070 (0.081)	3.85E-01	6.55E-01	0.040 (0.089)	6.52E-01	7.07E-01	-0.146 (0.093)	1.17E-01	4.67E-01	-0.215 (0.091)	1.88E-02	5.65E-02
PC aa C36:1	0.010 (0.070)	8.83E-01	7.30E-01	-0.064 (0.077)	4.13E-01	6.52E-01	-0.048 (0.063)	4.49E-01	7.32E-01	-0.062 (0.062)	3.19E-01	2.60E-01
PC aa C36:2	0.019 (0.080)	8.15E-01	7.05E-01	-0.020 (0.088)	8.17E-01	7.43E-01	-0.116 (0.075)	1.27E-01	4.67E-01	-0.157 (0.074)	3.55E-02	8.92E-02
PC aa C36:3	-0.007 (0.075)	9.25E-01	7.38E-01	-0.060 (0.083)	4.72E-01	6.63E-01	-0.045 (0.078)	5.64E-01	7.95E-01	-0.094 (0.076)	2.18E-01	2.14E-01
PC aa C36:4	0.127 (0.068)	6.26E-02	4.17E-01	0.161 (0.075)	3.30E-02	2.42E-01	-0.015 (0.058)	8.02E-01	8.52E-01	-0.008 (0.057)	8.81E-01	4.02E-01
PC aa C36:5	-0.033 (0.103)	7.47E-01	6.90E-01	-0.059 (0.114)	6.04E-01	7.07E-01	-0.156 (0.097)	1.10E-01	4.67E-01	-0.257 (0.095)	7.56E-03	**3.00E-02**
PC aa C36:6	0.038 (0.080)	6.33E-01	6.55E-01	-0.045 (0.089)	6.13E-01	7.07E-01	-0.063 (0.072)	3.86E-01	6.96E-01	-0.114 (0.071)	1.09E-01	1.57E-01
PC aa C38:0	0.009 (0.059)	8.76E-01	7.29E-01	0.007 (0.065)	9.16E-01	7.65E-01	-0.056 (0.063)	3.72E-01	6.96E-01	-0.087 (0.061)	1.57E-01	1.93E-01
PC aa C38:1	0.909 (0.411)	2.93E-02	3.68E-01	0.904 (0.427)	3.70E-02	2.47E-01	-0.683 (0.366)	6.45E-02	4.67E-01	0.024 (0.366)	9.48E-01	4.22E-01
PC aa C38:3	-0.010 (0.069)	8.87E-01	7.30E-01	-0.125 (0.077)	1.05E-01	4.06E-01	-0.018 (0.071)	8.02E-01	8.52E-01	-0.061 (0.069)	3.77E-01	2.77E-01
PC aa C38:4	0.139 (0.067)	3.96E-02	4.02E-01	0.128 (0.074)	8.61E-02	3.65E-01	0.018 (0.056)	7.45E-01	8.49E-01	0.032 (0.055)	5.58E-01	3.35E-01
PC aa C38:5	0.048 (0.065)	4.58E-01	6.55E-01	0.082 (0.071)	2.53E-01	5.59E-01	0.008 (0.055)	8.91E-01	8.76E-01	-0.002 (0.054)	9.72E-01	4.29E-01
PC aa C38:6	0.073 (0.074)	3.28E-01	6.55E-01	-0.070 (0.082)	3.96E-01	6.43E-01	-0.048 (0.077)	5.32E-01	7.88E-01	-0.108 (0.076)	1.56E-01	1.93E-01
PC aa C40:1	0.032 (0.055)	5.55E-01	6.55E-01	-0.021 (0.061)	7.30E-01	7.07E-01	-0.041 (0.058)	4.85E-01	7.47E-01	-0.079 (0.057)	1.71E-01	1.94E-01
PC aa C40:2	0.014 (0.090)	8.73E-01	7.29E-01	-0.134 (0.099)	1.79E-01	4.96E-01	0.035 (0.086)	6.85E-01	8.29E-01	0.031 (0.083)	7.09E-01	3.60E-01
PC aa C40:3	0.012 (0.062)	8.45E-01	7.17E-01	0.030 (0.069)	6.61E-01	7.07E-01	0.007 (0.057)	9.05E-01	8.76E-01	-0.022 (0.056)	6.97E-01	3.59E-01
PC aa C40:4	0.077 (0.063)	2.24E-01	6.55E-01	0.065 (0.070)	3.55E-01	6.07E-01	0.005 (0.064)	9.32E-01	8.76E-01	0.011 (0.062)	8.60E-01	3.97E-01
PC aa C40:5	0.045 (0.092)	6.27E-01	6.55E-01	0.032 (0.101)	7.50E-01	7.10E-01	0.063 (0.088)	4.75E-01	7.47E-01	0.075 (0.087)	3.90E-01	2.81E-01
PC aa C40:6	0.070 (0.084)	4.05E-01	6.55E-01	-0.137 (0.093)	1.44E-01	4.72E-01	-0.025 (0.081)	7.57E-01	8.49E-01	-0.083 (0.080)	3.02E-01	2.56E-01
PC aa C42:0	-0.007 (0.069)	9.23E-01	7.38E-01	-0.005 (0.077)	9.47E-01	7.71E-01	-0.010 (0.077)	8.95E-01	8.76E-01	-0.090 (0.075)	2.29E-01	2.21E-01
PC aa C42:1	0.051 (0.066)	4.43E-01	6.55E-01	0.005 (0.073)	9.50E-01	7.71E-01	0.011 (0.069)	8.71E-01	8.76E-01	-0.050 (0.067)	4.57E-01	3.07E-01
PC aa C42:2	0.114 (0.058)	5.14E-02	4.17E-01	0.087 (0.064)	1.78E-01	4.96E-01	-0.004 (0.061)	9.46E-01	8.77E-01	-0.058 (0.059)	3.30E-01	2.63E-01
PC aa C42:4	0.117 (0.055)	3.47E-02	3.83E-01	0.091 (0.061)	1.38E-01	4.60E-01	-0.020 (0.057)	7.26E-01	8.49E-01	0.016 (0.055)	7.79E-01	3.79E-01
PC aa C42:5	0.128 (0.068)	5.99E-02	4.17E-01	0.060 (0.075)	4.19E-01	6.52E-01	0.058 (0.064)	3.71E-01	6.96E-01	0.059 (0.063)	3.50E-01	2.69E-01
PC aa C42:6	0.103 (0.066)	1.22E-01	5.60E-01	0.056 (0.073)	4.43E-01	6.52E-01	0.018 (0.062)	7.76E-01	8.52E-01	0.021 (0.061)	7.36E-01	3.68E-01
PC ae C30:0	-0.016 (0.077)	8.34E-01	7.14E-01	-0.012 (0.085)	8.85E-01	7.57E-01	-0.178 (0.076)	2.04E-02	4.36E-01	-0.217 (0.074)	3.96E-03	**2.87E-02**
PC ae C30:1	0.762 (1.479)	6.12E-01	6.55E-01	0.576 (1.585)	7.20E-01	7.07E-01	2.076 (1.710)	2.53E-01	6.39E-01	2.509 (1.358)	9.44E-02	1.44E-01
PC ae C30:2	-0.011 (0.065)	8.68E-01	7.28E-01	0.044 (0.072)	5.41E-01	6.90E-01	-0.025 (0.062)	6.85E-01	8.29E-01	-0.067 (0.061)	2.69E-01	2.47E-01

PC ae C32:1	0.033 (0.083)	6.91E-01	6.67E-01	0.090 (0.092)	3.28E-01	5.78E-01	-0.133 (0.084)	1.12E-01	4.67E-01	-0.156 (0.082)	5.86E-02	1.22E-01
PC ae C32:2	0.041 (0.084)	6.24E-01	6.55E-01	0.061 (0.093)	5.15E-01	6.68E-01	-0.140 (0.080)	8.24E-02	4.67E-01	-0.148 (0.079)	6.18E-02	1.22E-01
PC ae C34:0	0.044 (0.093)	6.33E-01	6.55E-01	0.039 (0.103)	7.03E-01	7.07E-01	-0.161 (0.080)	4.38E-02	4.36E-01	-0.195 (0.078)	1.33E-02	**4.46E-02**
PC ae C34:1	0.038 (0.078)	6.27E-01	6.55E-01	0.028 (0.086)	7.41E-01	7.08E-01	-0.134 (0.078)	8.98E-02	4.67E-01	-0.132 (0.077)	8.82E-02	1.40E-01
PC ae C34:2	0.052 (0.075)	4.93E-01	6.55E-01	0.090 (0.083)	2.81E-01	5.78E-01	-0.079 (0.073)	2.81E-01	6.39E-01	-0.105 (0.071)	1.42E-01	1.84E-01
PC ae C34:3	0.047 (0.085)	5.79E-01	6.55E-01	0.092 (0.094)	3.27E-01	5.78E-01	-0.096 (0.087)	2.73E-01	6.39E-01	-0.168 (0.086)	5.07E-02	1.16E-01
PC ae C36:0	0.070 (0.071)	3.25E-01	6.55E-01	0.045 (0.079)	5.68E-01	7.00E-01	-0.035 (0.067)	5.99E-01	8.01E-01	-0.095 (0.066)	1.50E-01	1.90E-01
PC ae C36:1	0.041 (0.073)	5.69E-01	6.55E-01	0.016 (0.080)	8.42E-01	7.49E-01	-0.116 (0.069)	9.29E-02	4.67E-01	-0.129 (0.067)	5.61E-02	1.22E-01
PC ae C36:2	0.015 (0.080)	8.51E-01	7.17E-01	0.019 (0.088)	8.25E-01	7.43E-01	-0.123 (0.075)	1.06E-01	4.67E-01	-0.140 (0.074)	5.91E-02	1.22E-01
PC ae C36:3	-0.032 (0.079)	6.89E-01	6.67E-01	-0.031 (0.087)	7.26E-01	7.07E-01	-0.098 (0.079)	2.13E-01	6.20E-01	-0.142 (0.077)	6.78E-02	1.24E-01
PC ae C36:4	0.033 (0.066)	6.18E-01	6.55E-01	0.075 (0.073)	3.01E-01	5.78E-01	-0.060 (0.063)	3.43E-01	6.73E-01	-0.083 (0.062)	1.83E-01	1.97E-01
PC ae C36:5	0.101 (0.076)	1.88E-01	6.43E-01	0.166 (0.084)	5.12E-02	3.05E-01	-0.056 (0.073)	4.43E-01	7.27E-01	-0.077 (0.072)	2.83E-01	2.52E-01
PC ae C38:0	0.078 (0.070)	2.66E-01	6.55E-01	0.015 (0.077)	8.42E-01	7.49E-01	-0.065 (0.070)	3.52E-01	6.81E-01	-0.101 (0.068)	1.40E-01	1.84E-01
PC ae C38:1	-0.035 (0.121)	7.76E-01	6.95E-01	-0.008 (0.134)	9.51E-01	7.71E-01	-0.113 (0.100)	2.61E-01	6.39E-01	-0.034 (0.098)	7.28E-01	3.66E-01
PC ae C38:2	-0.125 (0.121)	3.02E-01	6.55E-01	-0.094 (0.134)	4.81E-01	6.68E-01	-0.170 (0.110)	1.26E-01	4.67E-01	-0.346 (0.108)	1.58E-03	**1.95E-02**
PC ae C38:3	-0.026 (0.072)	7.22E-01	6.84E-01	-0.101 (0.080)	2.07E-01	5.31E-01	-0.092 (0.069)	1.83E-01	5.73E-01	-0.123 (0.068)	7.04E-02	1.26E-01
PC ae C38:4	0.071 (0.066)	2.87E-01	6.55E-01	0.104 (0.073)	1.55E-01	4.89E-01	-0.064 (0.063)	3.09E-01	6.39E-01	-0.070 (0.062)	2.60E-01	2.45E-01
PC ae C38:5	0.063 (0.069)	3.64E-01	6.55E-01	0.137 (0.076)	7.47E-02	3.65E-01	-0.021 (0.066)	7.48E-01	8.49E-01	-0.026 (0.065)	6.83E-01	3.56E-01
PC ae C38:6	0.048 (0.070)	4.98E-01	6.55E-01	0.063 (0.078)	4.15E-01	6.52E-01	-0.091 (0.071)	2.00E-01	6.01E-01	-0.128 (0.069)	6.53E-02	1.22E-01
PC ae C40:1	0.106 (0.077)	1.66E-01	6.36E-01	0.029 (0.085)	7.31E-01	7.07E-01	-0.003 (0.069)	9.69E-01	8.85E-01	0.003 (0.068)	9.62E-01	4.26E-01
PC ae C40:2	0.003 (0.070)	9.66E-01	7.41E-01	-0.005 (0.078)	9.48E-01	7.71E-01	-0.085 (0.064)	1.87E-01	5.73E-01	-0.105 (0.063)	9.82E-02	1.48E-01
PC ae C40:3	0.051 (0.064)	4.25E-01	6.55E-01	0.011 (0.071)	8.72E-01	7.57E-01	-0.035 (0.063)	5.81E-01	7.95E-01	-0.079 (0.061)	2.00E-01	2.06E-01
PC ae C40:4	0.039 (0.072)	5.84E-01	6.55E-01	0.093 (0.079)	2.40E-01	5.59E-01	-0.032 (0.066)	6.24E-01	8.17E-01	-0.085 (0.064)	1.87E-01	1.97E-01
PC ae C40:5	0.041 (0.063)	5.18E-01	6.55E-01	0.079 (0.069)	2.56E-01	5.59E-01	-0.007 (0.062)	9.06E-01	8.76E-01	-0.013 (0.061)	8.27E-01	3.90E-01
PC ae C40:6	0.046 (0.070)	5.11E-01	6.55E-01	-0.026 (0.078)	7.40E-01	7.08E-01	-0.095 (0.071)	1.85E-01	5.73E-01	-0.130 (0.070)	6.39E-02	1.22E-01
PC ae C42:0	0.090 (0.053)	8.98E-02	5.03E-01	0.066 (0.059)	2.61E-01	5.59E-01	0.022 (0.054)	6.76E-01	8.29E-01	0.006 (0.052)	9.04E-01	4.09E-01
PC ae C42:1	0.073 (0.058)	2.12E-01	6.43E-01	0.086 (0.065)	1.85E-01	4.98E-01	0.023 (0.055)	6.77E-01	8.29E-01	0.039 (0.053)	4.67E-01	3.11E-01
PC ae C42:2	0.054 (0.052)	3.04E-01	6.55E-01	0.000 (0.058)	9.94E-01	7.76E-01	0.025 (0.053)	6.38E-01	8.20E-01	-0.022 (0.052)	6.71E-01	3.56E-01
PC ae C42:3	0.059 (0.062)	3.38E-01	6.55E-01	0.026 (0.068)	7.00E-01	7.07E-01	-0.008 (0.063)	8.98E-01	8.76E-01	-0.055 (0.062)	3.73E-01	2.77E-01

PC ae C42:4	0.055 (0.070)	4.37E-01	6.55E-01	0.102 (0.078)	1.92E-01	5.09E-01	0.000 (0.072)	1.00E+00	8.93E-01	-0.051 (0.071)	4.74E-01	3.12E-01
PC ae C42:5	0.015 (0.058)	8.00E-01	7.05E-01	0.030 (0.065)	6.44E-01	7.07E-01	0.013 (0.065)	8.47E-01	8.76E-01	-0.038 (0.063)	5.54E-01	3.35E-01
PC ae C44:3	0.032 (0.044)	4.63E-01	6.55E-01	0.006 (0.048)	9.08E-01	7.65E-01	0.087 (0.057)	1.26E-01	4.67E-01	0.087 (0.055)	1.20E-01	1.64E-01
PC ae C44:4	0.022 (0.065)	7.34E-01	6.86E-01	0.025 (0.072)	7.31E-01	7.07E-01	0.045 (0.063)	4.76E-01	7.47E-01	-0.013 (0.062)	8.30E-01	3.90E-01
PC ae C44:5	0.031 (0.075)	6.76E-01	6.67E-01	0.021 (0.082)	7.99E-01	7.34E-01	0.064 (0.079)	4.19E-01	7.06E-01	0.020 (0.077)	7.98E-01	3.84E-01
PC ae C44:6	0.032 (0.071)	6.51E-01	6.57E-01	0.041 (0.079)	6.00E-01	7.07E-01	0.030 (0.077)	6.98E-01	8.33E-01	-0.039 (0.076)	6.03E-01	3.35E-01
SM (OH) C14:1	0.024 (0.078)	7.64E-01	6.95E-01	0.005 (0.087)	9.57E-01	7.73E-01	-0.208 (0.069)	2.90E-03	2.45E-01	-0.276 (0.067)	6.48E-05	**3.99E-03**
SM (OH) C16:1	0.040 (0.072)	5.79E-01	6.55E-01	0.008 (0.080)	9.20E-01	7.66E-01	-0.204 (0.068)	2.89E-03	2.45E-01	-0.259 (0.066)	1.29E-04	**4.54E-03**
SM (OH) C22:1	-0.048 (0.078)	5.36E-01	6.55E-01	0.013 (0.086)	8.79E-01	7.57E-01	-0.183 (0.079)	2.12E-02	4.36E-01	-0.262 (0.077)	8.62E-04	**1.52E-02**
SM (OH) C22:2	-0.029 (0.084)	7.29E-01	6.84E-01	0.033 (0.093)	7.20E-01	7.07E-01	-0.176 (0.083)	3.48E-02	4.36E-01	-0.229 (0.081)	5.30E-03	**2.92E-02**
SM (OH) C24:1	-0.060 (0.076)	4.27E-01	6.55E-01	-0.029 (0.084)	7.28E-01	7.07E-01	-0.140 (0.070)	4.78E-02	4.36E-01	-0.189 (0.069)	6.64E-03	**2.92E-02**
SM C16:0	0.004 (0.064)	9.50E-01	7.41E-01	0.032 (0.071)	6.52E-01	7.07E-01	-0.105 (0.060)	8.17E-02	4.67E-01	-0.168 (0.059)	4.73E-03	**2.92E-02**
SM C16:1	-0.016 (0.076)	8.31E-01	7.14E-01	-0.017 (0.084)	8.35E-01	7.49E-01	-0.104 (0.067)	1.21E-01	4.67E-01	-0.185 (0.065)	5.11E-03	**2.92E-02**
SM C18:0	0.047 (0.068)	4.88E-01	6.55E-01	0.030 (0.075)	6.92E-01	7.07E-01	-0.106 (0.064)	1.01E-01	4.67E-01	-0.170 (0.063)	7.44E-03	**3.00E-02**
SM C18:1	0.034 (0.080)	6.69E-01	6.67E-01	-0.032 (0.088)	7.18E-01	7.07E-01	-0.086 (0.072)	2.38E-01	6.39E-01	-0.169 (0.071)	1.83E-02	5.63E-02
SM C20:2	0.113 (0.086)	1.90E-01	6.43E-01	0.101 (0.095)	2.86E-01	5.78E-01	-0.151 (0.074)	4.11E-02	4.36E-01	-0.220 (0.072)	2.59E-03	**2.28E-02**
SM C22:3	0.340 (0.110)	2.34E-03	1.25E-01	0.359 (0.121)	3.46E-03	6.89E-02	0.057 (0.115)	6.18E-01	8.14E-01	0.082 (0.113)	4.66E-01	3.11E-01
SM C24:0	-0.080 (0.061)	1.95E-01	6.43E-01	-0.094 (0.068)	1.65E-01	4.89E-01	-0.123 (0.064)	5.72E-02	4.67E-01	-0.174 (0.063)	6.08E-03	**2.92E-02**
SM C24:1	-0.018 (0.071)	7.99E-01	7.05E-01	0.012 (0.078)	8.76E-01	7.57E-01	-0.122 (0.066)	6.54E-02	4.67E-01	-0.166 (0.065)	1.08E-02	**3.98E-02**
SM C26:0	-0.030 (0.073)	6.79E-01	6.67E-01	0.099 (0.080)	2.21E-01	5.51E-01	-0.160 (0.073)	2.93E-02	4.36E-01	-0.221 (0.071)	2.22E-03	**2.10E-02**
SM C26:1	-0.031 (0.067)	6.44E-01	6.57E-01	-0.030 (0.074)	6.89E-01	7.07E-01	-0.138 (0.065)	3.51E-02	4.36E-01	-0.183 (0.064)	4.45E-03	**2.89E-02**

Ratios	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Acyl-carnitines												
(AC C16:0 + AC C18:0) / AC C0	-0.012 (0.125)	9.23E-01	7.38E-01	-0.148 (0.139)	2.86E-01	5.78E-01	-0.070 (0.115)	5.42E-01	7.88E-01	-0.037 (0.112)	7.46E-01	3.69E-01
(AC C2:0 + AC C3:0) / AC C0	-0.001 (0.061)	9.88E-01	7.49E-01	0.005 (0.068)	9.36E-01	7.71E-01	-0.095 (0.056)	9.06E-02	4.67E-01	-0.029 (0.055)	6.01E-01	3.35E-01
AC C12:0 / AC C10:0	-0.046 (0.071)	5.21E-01	6.55E-01	-0.095 (0.078)	2.25E-01	5.51E-01	-0.011 (0.067)	8.69E-01	8.76E-01	-0.012 (0.066)	8.50E-01	3.96E-01

AC C14:0 / AC C16:1	-0.125 (0.050)	1.44E-02	2.87E-01	-0.232 (0.056)	4.88E-05	**5.38E-03**	-0.029 (0.051)	5.73E-01	7.95E-01	-0.149 (0.050)	3.15E-03	**2.43E-02**
AC C2:0 / AC C0	-0.013 (0.066)	8.46E-01	7.17E-01	-0.025 (0.072)	7.35E-01	7.08E-01	-0.095 (0.062)	1.29E-01	4.67E-01	-0.051 (0.061)	4.06E-01	2.87E-01
AC C3:0 / AC C4:0	0.013 (0.128)	9.20E-01	7.38E-01	0.113 (0.142)	4.27E-01	6.52E-01	-0.033 (0.111)	7.65E-01	8.49E-01	0.202 (0.109)	6.45E-02	1.22E-01
Amino acids												
Cit / Arg	-0.134 (0.158)	3.96E-01	6.55E-01	0.153 (0.174)	3.80E-01	6.30E-01	0.311 (0.153)	4.39E-02	4.36E-01	0.539 (0.150)	4.18E-04	**1.03E-02**
Cit / Orn	-0.025 (0.061)	6.82E-01	6.67E-01	0.073 (0.068)	2.84E-01	5.78E-01	-0.015 (0.055)	7.91E-01	8.52E-01	0.031 (0.054)	5.74E-01	3.35E-01
Fisher	0.062 (0.047)	1.87E-01	6.43E-01	0.096 (0.052)	6.45E-02	3.31E-01	-0.001 (0.041)	9.85E-01	8.89E-01	0.017 (0.041)	6.76E-01	3.56E-01
Gly / PC ae C38:2	0.049 (0.126)	6.98E-01	6.70E-01	-0.080 (0.139)	5.67E-01	7.00E-01	0.094 (0.109)	3.88E-01	6.96E-01	0.234 (0.107)	2.97E-02	7.94E-02
Orn / Arg	-0.109 (0.159)	4.92E-01	6.55E-01	0.081 (0.175)	6.45E-01	7.07E-01	0.326 (0.160)	4.36E-02	4.36E-01	0.509 (0.157)	1.40E-03	**1.95E-02**
Orn / Ser	0.055 (0.058)	3.42E-01	6.55E-01	0.100 (0.064)	1.17E-01	4.32E-01	0.111 (0.063)	8.10E-02	4.67E-01	0.115 (0.062)	6.46E-02	1.22E-01
Tyr / Phe	0.051 (0.036)	1.63E-01	6.36E-01	0.039 (0.040)	3.28E-01	5.78E-01	-0.002 (0.036)	9.54E-01	8.80E-01	0.022 (0.035)	5.36E-01	3.32E-01
Bile acids												
Conj. BA / unconj. BA	-0.184 (0.254)	4.70E-01	6.55E-01	-0.210 (0.280)	4.54E-01	6.58E-01	-0.074 (0.238)	7.58E-01	8.49E-01	-0.107 (0.233)	6.47E-01	3.51E-01
Prim. BA / sec. BA	-0.134 (0.245)	5.87E-01	6.55E-01	-0.273 (0.271)	3.15E-01	5.78E-01	0.029 (0.248)	9.08E-01	8.76E-01	-0.005 (0.242)	9.84E-01	4.30E-01
Phospholipids												
LysoPC / PC	-0.036 (0.059)	5.43E-01	6.55E-01	-0.073 (0.065)	2.64E-01	5.59E-01	0.068 (0.063)	2.85E-01	6.39E-01	0.041 (0.062)	5.10E-01	3.22E-01
MUFA PC / SFA PC	-0.036 (0.035)	3.09E-01	6.55E-01	-0.075 (0.039)	5.83E-02	3.21E-01	0.035 (0.035)	3.16E-01	6.39E-01	0.058 (0.034)	8.90E-02	1.40E-01
PC aa C36:3 / PC aa C36:4	-0.134 (0.070)	5.67E-02	4.17E-01	-0.221 (0.077)	4.78E-03	7.54E-02	-0.030 (0.071)	6.68E-01	8.29E-01	-0.086 (0.070)	2.19E-01	2.14E-01
PC aa C40:3 / PC aa C42:5	-0.116 (0.061)	5.92E-02	4.17E-01	-0.030 (0.067)	6.55E-01	7.07E-01	-0.051 (0.059)	3.93E-01	6.96E-01	-0.081 (0.058)	1.66E-01	1.93E-01
PC ae C32:1 / PC ae C34:1	-0.005 (0.042)	9.10E-01	7.38E-01	0.062 (0.046)	1.80E-01	4.96E-01	0.000 (0.035)	9.96E-01	8.93E-01	-0.024 (0.034)	4.82E-01	3.14E-01
PC ae C38:1 / PC aa C28:1	-0.038 (0.108)	7.25E-01	6.84E-01	0.042 (0.120)	7.30E-01	7.07E-01	-0.038 (0.091)	6.78E-01	8.29E-01	0.111 (0.089)	2.12E-01	2.14E-01
PC ae C44:5 / PC ae C42:5	0.016 (0.031)	5.97E-01	6.55E-01	-0.009 (0.034)	7.97E-01	7.34E-01	0.051 (0.028)	6.72E-02	4.67E-01	0.057 (0.027)	3.66E-02	9.01E-02
PUFA-PC / MUFA-PC	0.036 (0.039)	3.58E-01	6.55E-01	0.053 (0.043)	2.22E-01	5.51E-01	0.004 (0.037)	9.16E-01	8.76E-01	-0.013 (0.036)	7.15E-01	3.61E-01
PUFA-PC / SFA-PC	0.000 (0.030)	1.00E+00	7.53E-01	-0.022 (0.033)	5.21E-01	6.68E-01	0.039 (0.034)	2.63E-01	6.39E-01	0.045 (0.034)	1.88E-01	1.97E-01
SM (OH) C24:1 / SM C16:0	-0.064 (0.056)	2.51E-01	6.55E-01	-0.061 (0.062)	3.23E-01	5.78E-01	-0.035 (0.048)	4.68E-01	7.47E-01	-0.021 (0.047)	6.54E-01	3.52E-01
Sums	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**
Acyl-carnitines												

AC C16 + AC C18	0.102 (0.127)	4.25E-01	6.55E-01	0.019 (0.141)	8.92E-01	7.57E-01	0.031 (0.117)	7.92E-01	8.52E-01	0.060 (0.114)	6.03E-01	3.35E-01
AC C2 + AC C3	0.113 (0.089)	2.07E-01	6.43E-01	0.173 (0.098)	8.10E-02	3.65E-01	-0.009 (0.079)	9.07E-01	8.76E-01	0.053 (0.078)	4.99E-01	3.20E-01
Carnitines	0.104 (0.081)	2.00E-01	6.43E-01	0.156 (0.089)	8.21E-02	3.65E-01	-0.016 (0.073)	8.29E-01	8.74E-01	0.057 (0.072)	4.24E-01	2.95E-01
Even. carn	0.108 (0.085)	2.03E-01	6.43E-01	0.146 (0.094)	1.22E-01	4.35E-01	-0.013 (0.078)	8.66E-01	8.76E-01	0.049 (0.077)	5.26E-01	3.27E-01
Long. carn	0.100 (0.124)	4.22E-01	6.55E-01	0.077 (0.137)	5.75E-01	7.01E-01	0.032 (0.113)	7.79E-01	8.52E-01	0.103 (0.111)	3.58E-01	2.70E-01
Medium. carn	0.134 (0.103)	1.93E-01	6.43E-01	0.099 (0.113)	3.85E-01	6.34E-01	-0.046 (0.096)	6.37E-01	8.20E-01	0.036 (0.094)	7.01E-01	3.59E-01
Odd. carn	0.073 (0.082)	3.75E-01	6.55E-01	0.158 (0.091)	8.29E-02	3.65E-01	-0.042 (0.073)	5.67E-01	7.95E-01	0.095 (0.071)	1.83E-01	1.97E-01
Amino acids												
AA	-0.003 (0.032)	9.30E-01	7.39E-01	-0.041 (0.036)	2.57E-01	5.59E-01	-0.050 (0.030)	1.01E-01	4.67E-01	-0.060 (0.030)	4.38E-02	1.04E-01
Arom. AA	0.074 (0.034)	3.31E-02	3.83E-01	0.060 (0.038)	1.14E-01	4.32E-01	-0.020 (0.034)	5.62E-01	7.95E-01	-0.006 (0.033)	8.64E-01	3.97E-01
BCAA	0.136 (0.052)	9.39E-03	2.86E-01	0.157 (0.057)	6.81E-03	7.91E-02	-0.019 (0.044)	6.60E-01	8.29E-01	0.021 (0.043)	6.27E-01	3.45E-01
Ess. AA	0.051 (0.035)	1.49E-01	6.35E-01	0.035 (0.039)	3.78E-01	6.30E-01	-0.031 (0.030)	3.06E-01	6.39E-01	-0.030 (0.030)	3.18E-01	2.60E-01
Glucog. AA	-0.060 (0.044)	1.67E-01	6.36E-01	-0.134 (0.048)	5.75E-03	7.91E-02	-0.047 (0.048)	3.29E-01	6.55E-01	-0.083 (0.047)	7.91E-02	1.35E-01
Noness. AA	-0.038 (0.035)	2.89E-01	6.55E-01	-0.095 (0.039)	1.62E-02	1.49E-01	-0.064 (0.037)	8.56E-02	4.67E-01	-0.084 (0.036)	2.19E-02	6.14E-02
Bile acids												
Conj. BA	0.125 (0.174)	4.72E-01	6.55E-01	0.108 (0.192)	5.74E-01	7.01E-01	-0.259 (0.165)	1.18E-01	4.67E-01	-0.116 (0.161)	4.71E-01	3.12E-01
Prim. BA	0.114 (0.170)	5.02E-01	6.55E-01	0.122 (0.188)	5.17E-01	6.68E-01	-0.249 (0.162)	1.25E-01	4.67E-01	-0.103 (0.158)	5.17E-01	3.23E-01
Sec. BA	0.240 (0.226)	2.89E-01	6.55E-01	0.356 (0.250)	1.56E-01	4.89E-01	-0.190 (0.230)	4.10E-01	7.01E-01	0.017 (0.226)	9.41E-01	4.21E-01
Unconj. BA	0.123 (0.165)	4.57E-01	6.55E-01	0.255 (0.183)	1.65E-01	4.89E-01	0.011 (0.169)	9.47E-01	8.77E-01	0.089 (0.166)	5.92E-01	3.35E-01
Phospholipids												
Long. PC	0.058 (0.066)	3.79E-01	6.55E-01	-0.053 (0.073)	4.64E-01	6.58E-01	-0.015 (0.064)	8.12E-01	8.59E-01	-0.055 (0.063)	3.80E-01	2.77E-01
Long. PC aa	0.064 (0.074)	3.95E-01	6.55E-01	-0.087 (0.082)	2.94E-01	5.78E-01	-0.013 (0.072)	8.55E-01	8.76E-01	-0.053 (0.071)	4.53E-01	3.07E-01
Long. PC ae	0.045 (0.057)	4.32E-01	6.55E-01	0.036 (0.062)	5.67E-01	7.00E-01	-0.023 (0.057)	6.94E-01	8.33E-01	-0.057 (0.056)	3.13E-01	2.60E-01
Long. SM	-0.039 (0.065)	5.46E-01	6.55E-01	-0.021 (0.072)	7.67E-01	7.19E-01	-0.124 (0.063)	5.00E-02	4.36E-01	-0.170 (0.062)	6.37E-03	**2.92E-02**
Long. SM C	-0.039 (0.065)	5.53E-01	6.55E-01	-0.021 (0.072)	7.70E-01	7.19E-01	-0.124 (0.063)	5.12E-02	4.36E-01	-0.170 (0.062)	6.57E-03	**2.92E-02**
Long. SM OH	-0.060 (0.076)	4.27E-01	6.55E-01	-0.029 (0.084)	7.28E-01	7.07E-01	-0.140 (0.070)	4.78E-02	4.36E-01	-0.189 (0.069)	6.64E-03	**2.92E-02**
LysoPC	0.032 (0.061)	5.96E-01	6.55E-01	-0.028 (0.067)	6.81E-01	7.07E-01	0.035 (0.062)	5.72E-01	7.95E-01	-0.005 (0.061)	9.33E-01	4.19E-01
Mono. PC	0.038 (0.071)	5.95E-01	6.55E-01	0.000 (0.078)	9.96E-01	7.76E-01	-0.063 (0.068)	3.56E-01	6.81E-01	-0.063 (0.066)	3.41E-01	2.66E-01

Mono. PC aa	0.037 (0.071)	6.05E-01	6.55E-01	-0.003 (0.079)	9.69E-01	7.76E-01	-0.059 (0.068)	3.89E-01	6.96E-01	-0.059 (0.067)	3.75E-01	2.77E-01
Mono. PC ae	0.042 (0.070)	5.44E-01	6.55E-01	0.038 (0.077)	6.26E-01	7.07E-01	-0.115 (0.069)	9.77E-02	4.67E-01	-0.116 (0.068)	8.74E-02	1.40E-01
PC	0.068 (0.059)	2.52E-01	6.55E-01	0.045 (0.065)	4.95E-01	6.68E-01	-0.061 (0.056)	2.83E-01	6.39E-01	-0.076 (0.055)	1.72E-01	1.94E-01
PC aa	0.070 (0.059)	2.43E-01	6.55E-01	0.042 (0.066)	5.20E-01	6.68E-01	-0.060 (0.056)	2.91E-01	6.39E-01	-0.074 (0.055)	1.81E-01	1.97E-01
PC ae	0.048 (0.063)	4.51E-01	6.55E-01	0.071 (0.070)	3.07E-01	5.78E-01	-0.073 (0.062)	2.41E-01	6.39E-01	-0.094 (0.061)	1.22E-01	1.66E-01
Poly. PC	0.074 (0.059)	2.11E-01	6.43E-01	0.053 (0.065)	4.18E-01	6.52E-01	-0.059 (0.056)	2.92E-01	6.39E-01	-0.077 (0.055)	1.62E-01	1.93E-01
Poly. PC aa	0.076 (0.059)	1.99E-01	6.43E-01	0.050 (0.065)	4.41E-01	6.52E-01	-0.058 (0.056)	2.97E-01	6.39E-01	-0.076 (0.054)	1.67E-01	1.93E-01
Poly. PC ae	0.047 (0.063)	4.52E-01	6.55E-01	0.078 (0.069)	2.60E-01	5.59E-01	-0.066 (0.062)	2.88E-01	6.39E-01	-0.089 (0.061)	1.42E-01	1.84E-01
Sat. LysoPC	0.037 (0.065)	5.65E-01	6.55E-01	-0.033 (0.071)	6.44E-01	7.07E-01	0.053 (0.069)	4.39E-01	7.27E-01	-0.002 (0.067)	9.79E-01	4.30E-01
Sat. PC	0.074 (0.068)	2.82E-01	6.55E-01	0.074 (0.076)	3.27E-01	5.78E-01	-0.098 (0.068)	1.50E-01	5.14E-01	-0.121 (0.066)	6.85E-02	1.24E-01
Sat. PC aa	0.075 (0.071)	2.94E-01	6.55E-01	0.084 (0.079)	2.90E-01	5.78E-01	-0.098 (0.070)	1.62E-01	5.26E-01	-0.120 (0.069)	8.25E-02	1.35E-01
Sat. PC ae	0.065 (0.066)	3.25E-01	6.55E-01	0.029 (0.073)	6.97E-01	7.07E-01	-0.085 (0.063)	1.80E-01	5.73E-01	-0.121 (0.062)	5.07E-02	1.16E-01
Saturmono. PC	0.038 (0.071)	5.95E-01	6.55E-01	0.000 (0.078)	9.96E-01	7.76E-01	-0.063 (0.068)	3.56E-01	6.81E-01	-0.063 (0.066)	3.41E-01	2.66E-01
Short. PC	0.044 (0.074)	5.49E-01	6.55E-01	0.035 (0.082)	6.69E-01	7.07E-01	-0.080 (0.072)	2.67E-01	6.39E-01	-0.088 (0.071)	2.13E-01	2.14E-01
Short. PC aa	0.044 (0.074)	5.52E-01	6.55E-01	0.034 (0.082)	6.83E-01	7.07E-01	-0.079 (0.072)	2.77E-01	6.39E-01	-0.086 (0.071)	2.24E-01	2.18E-01
Short. PC ae	0.043 (0.074)	5.63E-01	6.55E-01	0.063 (0.082)	4.46E-01	6.52E-01	-0.138 (0.076)	7.06E-02	4.67E-01	-0.158 (0.075)	3.55E-02	8.92E-02
SM	0.001 (0.065)	9.92E-01	7.50E-01	0.010 (0.072)	8.85E-01	7.57E-01	-0.113 (0.061)	6.63E-02	4.67E-01	-0.173 (0.060)	4.37E-03	**2.89E-02**
SM (OH)	-0.018 (0.075)	8.13E-01	7.05E-01	0.016 (0.083)	8.48E-01	7.52E-01	-0.186 (0.072)	1.08E-02	4.36E-01	-0.247 (0.071)	6.11E-04	**1.25E-02**
SM C	0.002 (0.065)	9.72E-01	7.41E-01	0.010 (0.072)	8.88E-01	7.57E-01	-0.107 (0.061)	8.06E-02	4.67E-01	-0.168 (0.060)	5.62E-03	**2.92E-02**
Unsat. lysoPC	0.010 (0.069)	8.85E-01	7.30E-01	-0.018 (0.077)	8.20E-01	7.43E-01	-0.008 (0.064)	9.01E-01	8.76E-01	-0.015 (0.063)	8.12E-01	3.86E-01
Very. lysoPC	0.044 (0.032)	1.64E-01	6.36E-01	0.035 (0.035)	3.16E-01	5.78E-01	0.028 (0.029)	3.41E-01	6.73E-01	0.036 (0.029)	2.16E-01	2.14E-01

Data are β-estimate (SE) of UCB metabolite concentrations, sums and ratios in offspring of obese and offspring of severely obese mothers in reference to offspring of normal weight mothers after stratification for sex. Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear regression models adjusted for batch, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed in n = 111 offspring of normal weight, n = 128 offspring of obese, and n = 159 offspring of severely obese mother. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. GA, gestational age; GWG, gestational weight gain; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error; TG, triglyceride; UCB, umbilical cord blood

Table S 7: Beta-comparison of β-estimates of the metabolite concentrations that were significantly associated with maternal severe obesity between male and female offspring

	Male offspring of severely obese vs. offspring of normal weight mothers			Female offspring of severely obese vs. offspring of normal weight mothers			
Metabolite	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-estimate (SE)**	**p-Value**	**q-Value**	**β-compare**
Arg	-0.143 (0.157)	3.66E-01	6.21E-01	-0.573 (0.137)	4.57E-05	**3.99E-03**	4.04E-02
Betaine	0.299 (0.081)	2.97E-04	**1.60E-02**	-0.011 (0.059)	8.52E-01	3.96E-01	2.21E-03
PC aa C30:0	0.039 (0.084)	6.48E-01	7.07E-01	-0.192 (0.070)	6.91E-03	**2.93E-02**	3.64E-02
SM (OH) C14:1	0.005 (0.087)	9.57E-01	7.73E-01	-0.276 (0.067)	6.48E-05	**3.99E-03**	1.13E-02
SM (OH) C16:1	0.008 (0.080)	9.20E-01	7.66E-01	-0.259 (0.066)	1.29E-04	**4.54E-03**	1.07E-02
SM (OH) C22:1	0.013 (0.086)	8.79E-01	7.57E-01	-0.262 (0.077)	8.62E-04	**1.52E-02**	1.81E-02
SM (OH) C22:2	0.033 (0.093)	7.20E-01	7.07E-01	-0.229 (0.081)	5.30E-03	**2.92E-02**	3.43E-02
SM C16:0	0.032 (0.071)	6.52E-01	7.07E-01	-0.168 (0.059)	4.73E-03	**2.92E-02**	3.09E-02
SM C18:0	0.030 (0.075)	6.92E-01	7.07E-01	-0.170 (0.063)	7.44E-03	**3.00E-02**	4.17E-02
SM C20:2	0.101 (0.095)	2.86E-01	5.78E-01	-0.220 (0.072)	2.59E-03	**2.28E-02**	7.36E-03
SM C26:0	0.099 (0.080)	2.21E-01	5.51E-01	-0.221 (0.071)	2.22E-03	**2.10E-02**	3.15E-03

Data are β-estimate (SE) of UCB metabolite concentrations (µmol/L) of significant associations of male and female offspring of severely obese mothers in reference to offspring of normal weight mothers as provided in Table S 6 and the comparison thereof. Data are based on linear regression models adjusted for batch, offspring sex, GA, birth weight, mode of delivery, GWG and maternal HbA1c (%) at delivery performed n = 60 female offspring of normal weight mothers, n = 65 female offspring of obese, n = 86 female offspring of severely obese, n = 51 male offspring of normal weight, n = 63 male offspring of obese, and 79 offspring of severely obese mothers after the dataset was stratified by offspring sex. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates q-value (p-value adjusted for multiple testing) < 0.05. A full list of metabolite abbreviations is provided in Table S 1. GA, gestational age; GWG, gestational weight gain; SE, standard error; UCB, umbilical cord blood.

Table S 8: Association of late-pregnancy dysglycemia in obese women and UCB metabolites

Metabolite	β-estimate (SE)	p-Value
Acyl-carnitines		
AC 2-M-C3:0	0.023 (0.092)	8.02E-01
AC 2-M-C3:1	0.109 (0.125)	3.86E-01
AC 2-M-C4:0	-0.009 (0.098)	9.26E-01
AC 3-M-C4:0	-0.029 (0.167)	8.60E-01
AC C0	-0.012 (0.049)	8.03E-01
AC C10:0	0.136 (0.093)	1.43E-01
AC C10:1	0.159 (0.081)	5.12E-02
AC C11:0	0.086 (0.105)	4.12E-01
AC C12:0	0.241 (0.096)	1.29E-02
AC C12:1	0.333 (0.094)	4.58E-04
AC C13:0	0.156 (0.099)	1.17E-01
AC C14:0	0.369 (0.110)	9.19E-04
AC C14:1	0.331 (0.106)	2.08E-03
AC C15:0	0.245 (0.115)	3.40E-02
AC C16:0	0.288 (0.108)	7.95E-03
AC C16:1	0.379 (0.114)	9.60E-04
AC C17:0	0.171 (0.099)	8.51E-02
AC C18:0	0.195 (0.092)	3.57E-02
AC C18:1	0.354 (0.104)	7.60E-04
AC C18:2	0.319 (0.116)	6.47E-03
AC C2:0	0.039 (0.072)	5.90E-01
AC C3:0	-0.037 (0.091)	6.87E-01
AC C3-DC	0.126 (0.060)	3.59E-02
AC C3-M-DC	0.034 (0.064)	5.91E-01
AC C4:0	0.022 (0.088)	8.00E-01
AC C4:1	0.254 (0.215)	2.40E-01
AC C4-DC	0.182 (0.093)	5.17E-02
AC C4-OHa	0.130 (0.131)	3.22E-01
AC C4-OHb	0.150 (0.190)	4.32E-01
AC C5:1	-0.022 (0.099)	8.26E-01
AC C5-DC	0.050 (0.072)	4.87E-01
AC C5-M-DC	0.056 (0.177)	7.54E-01
AC C5-OH	0.013 (0.078)	8.66E-01
AC C6:0	-0.097 (0.120)	4.21E-01
AC C6:1	0.126 (0.102)	2.19E-01
AC C6-DC	0.142 (0.155)	3.59E-01
AC C6-OHa	0.128 (0.119)	2.86E-01
AC C6-OHb	0.051 (0.097)	6.03E-01
AC C7:0	0.024 (0.089)	7.85E-01
AC C7-DC	0.057 (0.093)	5.43E-01

AC C8:0	0.052 (0.097)	5.94E-01
AC C8:1	0.130 (0.135)	3.36E-01
AC C8-OH	0.207 (0.089)	2.04E-02
AC C9:0	0.035 (0.115)	7.63E-01
AC iso-C11:0	0.015 (0.096)	8.77E-01
AC iso-C13:0	0.031 (0.126)	8.08E-01
AC iso-C15:0	0.180 (0.101)	7.45E-02
AC iso-C17:0	0.169 (0.103)	1.03E-01
AC iso-C9:0	0.088 (0.113)	4.34E-01
Amino acids		
1-M-His	0.198 (0.073)	7.23E-03
3-M-His	-0.144 (0.306)	6.39E-01
AAB	-0.117 (0.087)	1.78E-01
AADP	0.175 (0.077)	2.46E-02
Ala	0.076 (0.049)	1.27E-01
Anserine	0.050 (0.258)	8.46E-01
Arg	-0.258 (0.121)	3.39E-02
Asn	-0.048 (0.046)	3.00E-01
Asp	0.054 (0.066)	4.11E-01
BAIB	-0.045 (0.142)	7.52E-01
Carnosine	0.152 (0.251)	5.46E-01
Cit	-0.044 (0.052)	3.97E-01
Cys-Cys	0.112 (0.071)	1.15E-01
Dimethylglycine	0.029 (0.071)	6.83E-01
GABA	0.268 (0.125)	3.24E-02
Gln	-0.085 (0.051)	9.98E-02
Glu	0.076 (0.056)	1.74E-01
Gly	-0.034 (0.040)	3.89E-01
Hcys	0.261 (0.130)	4.58E-02
His	-0.068 (0.046)	1.38E-01
Ile	-0.014 (0.054)	7.91E-01
Leu	-0.104 (0.055)	6.05E-02
Lys	0.054 (0.035)	1.23E-01
Met	-0.024 (0.033)	4.69E-01
OH-Pro	0.007 (0.040)	8.60E-01
Orn	-0.040 (0.042)	3.42E-01
Phe	-0.016 (0.032)	6.17E-01
Pro	-0.001 (0.032)	9.76E-01
Sarcosine	0.063 (0.070)	3.67E-01
Ser	0.064 (0.046)	1.65E-01
Thr	-0.021 (0.048)	6.70E-01
Trp	-0.070 (0.042)	9.90E-02
Tyr	-0.013 (0.035)	7.18E-01
Val	-0.062 (0.037)	9.26E-02

β-Ala	0.033 (0.105)	7.56E-01
Amino acid derivatives		
Betaine	0.062 (0.059)	2.89E-01
Choline	0.037 (0.051)	4.71E-01
Bile acids		
CA	0.073 (0.163)	6.56E-01
CDCA	0.090 (0.093)	3.34E-01
DCA	-0.064 (0.270)	8.13E-01
GCA	-0.040 (0.167)	8.13E-01
GCDCA	-0.106 (0.169)	5.31E-01
GDCA	-0.495 (0.245)	4.46E-02
GLCA	-0.468 (0.392)	2.35E-01
GUDCA	-0.200 (0.238)	4.01E-01
LCA	-0.665 (0.338)	5.12E-02
TCA	0.078 (0.168)	6.45E-01
TCDCA	0.012 (0.148)	9.35E-01
TDCA	-0.196 (0.271)	4.70E-01
TLCA	0.054 (0.249)	8.28E-01
TLCA-S	0.126 (0.151)	4.03E-01
TUDCA	0.198 (0.157)	2.10E-01
UDCA	0.188 (0.233)	4.21E-01
Lipids		
HDL cholesterol	-0.067 (0.064)	2.96E-01
LDL cholesterol	-0.059 (0.072)	4.16E-01
TG	0.126 (0.098)	1.98E-01
Phospholipids		
LysoPC a C14:0	0.122 (0.052)	1.89E-02
LysoPC a C16:0	0.112 (0.057)	5.16E-02
LysoPC a C16:1	0.118 (0.060)	4.97E-02
LysoPC a C17:0	-0.015 (0.052)	7.72E-01
LysoPC a C18:0	0.046 (0.067)	4.91E-01
LysoPC a C18:1	0.079 (0.055)	1.53E-01
LysoPC a C18:2	0.093 (0.071)	1.94E-01
LysoPC a C18:3	0.088 (0.073)	2.27E-01
LysoPC a C20:3	0.084 (0.069)	2.24E-01
LysoPC a C20:4	0.066 (0.070)	3.48E-01
LysoPC a C20:5	0.136 (0.093)	1.46E-01
LysoPC a C22:5	0.032 (0.092)	7.31E-01
LysoPC a C22:6	-0.012 (0.080)	8.80E-01
LysoPC a C24:0	-0.043 (0.047)	3.58E-01
LysoPC a C26:0	-0.066 (0.078)	3.95E-01
LysoPC a C26:1	-0.016 (0.022)	4.75E-01
LysoPC a C28:0	-0.057 (0.035)	1.03E-01
LysoPC a C28:1	-0.070 (0.068)	3.02E-01

PC aa C24:0	0.008 (0.071)	9.15E-01
PC aa C26:0	0.015 (0.045)	7.37E-01
PC aa C28:1	0.040 (0.058)	4.86E-01
PC aa C30:0	0.036 (0.064)	5.72E-01
PC aa C32:0	0.002 (0.067)	9.77E-01
PC aa C32:1	0.010 (0.104)	9.27E-01
PC aa C32:3	-0.047 (0.058)	4.18E-01
PC aa C34:1	-0.006 (0.059)	9.23E-01
PC aa C34:2	0.041 (0.073)	5.79E-01
PC aa C34:3	-0.039 (0.083)	6.41E-01
PC aa C34:4	-0.041 (0.063)	5.10E-01
PC aa C36:0	-0.066 (0.074)	3.74E-01
PC aa C36:1	-0.030 (0.057)	5.98E-01
PC aa C36:2	0.015 (0.067)	8.24E-01
PC aa C36:3	0.052 (0.063)	4.09E-01
PC aa C36:4	-0.015 (0.053)	7.80E-01
PC aa C36:5	0.010 (0.080)	9.02E-01
PC aa C36:6	-0.051 (0.064)	4.24E-01
PC aa C38:0	-0.026 (0.051)	6.09E-01
PC aa C38:1	-0.136 (0.290)	6.40E-01
PC aa C38:3	0.021 (0.059)	7.28E-01
PC aa C38:4	-0.062 (0.053)	2.44E-01
PC aa C38:5	-0.031 (0.050)	5.30E-01
PC aa C38:6	-0.091 (0.065)	1.61E-01
PC aa C40:1	-0.089 (0.049)	7.04E-02
PC aa C40:2	0.001 (0.075)	9.92E-01
PC aa C40:3	0.030 (0.049)	5.36E-01
PC aa C40:4	-0.074 (0.056)	1.87E-01
PC aa C40:5	-0.056 (0.076)	4.61E-01
PC aa C40:6	-0.125 (0.072)	8.45E-02
PC aa C42:0	-0.180 (0.064)	5.36E-03
PC aa C42:1	-0.117 (0.059)	4.71E-02
PC aa C42:2	-0.052 (0.051)	3.13E-01
PC aa C42:4	-0.062 (0.048)	2.02E-01
PC aa C42:5	-0.034 (0.058)	5.65E-01
PC aa C42:6	-0.083 (0.055)	1.34E-01
PC ae C30:0	0.040 (0.064)	5.30E-01
PC ae C30:1	-0.177 (0.799)	8.26E-01
PC ae C30:2	-0.056 (0.055)	3.08E-01
PC ae C32:1	-0.009 (0.070)	9.02E-01
PC ae C32:2	-0.020 (0.070)	7.76E-01
PC ae C34:0	-0.086 (0.070)	2.22E-01
PC ae C34:1	0.008 (0.066)	8.99E-01
PC ae C34:2	-0.015 (0.063)	8.08E-01

PC ae C34:3	-0.124 (0.075)	9.70E-02
PC ae C36:0	-0.027 (0.058)	6.40E-01
PC ae C36:1	-0.073 (0.058)	2.11E-01
PC ae C36:2	-0.050 (0.066)	4.49E-01
PC ae C36:3	0.028 (0.066)	6.69E-01
PC ae C36:4	0.016 (0.055)	7.74E-01
PC ae C36:5	-0.026 (0.062)	6.81E-01
PC ae C38:0	-0.081 (0.057)	1.55E-01
PC ae C38:1	-0.147 (0.094)	1.19E-01
PC ae C38:2	-0.033 (0.094)	7.26E-01
PC ae C38:3	-0.041 (0.058)	4.78E-01
PC ae C38:4	-0.063 (0.055)	2.56E-01
PC ae C38:5	-0.004 (0.057)	9.46E-01
PC ae C38:6	-0.038 (0.058)	5.14E-01
PC ae C40:1	0.065 (0.062)	2.98E-01
PC ae C40:2	-0.027 (0.057)	6.33E-01
PC ae C40:3	-0.053 (0.052)	3.10E-01
PC ae C40:4	-0.059 (0.059)	3.19E-01
PC ae C40:5	-0.053 (0.053)	3.18E-01
PC ae C40:6	-0.135 (0.059)	2.39E-02
PC ae C42:0	-0.043 (0.044)	3.29E-01
PC ae C42:1	-0.048 (0.048)	3.12E-01
PC ae C42:2	-0.027 (0.045)	5.49E-01
PC ae C42:3	-0.039 (0.052)	4.56E-01
PC ae C42:4	-0.083 (0.062)	1.85E-01
PC ae C42:5	-0.088 (0.053)	9.59E-02
PC ae C44:3	0.036 (0.042)	3.96E-01
PC ae C44:4	-0.067 (0.054)	2.16E-01
PC ae C44:5	-0.123 (0.066)	6.46E-02
PC ae C44:6	-0.125 (0.065)	5.38E-02
SM (OH) C14:1	-0.029 (0.063)	6.47E-01
SM (OH) C16:1	-0.023 (0.060)	7.02E-01
SM (OH) C22:1	0.063 (0.065)	3.28E-01
SM (OH) C22:2	0.053 (0.070)	4.47E-01
SM (OH) C24:1	0.047 (0.060)	4.37E-01
SM C16:0	0.018 (0.053)	7.37E-01
SM C16:1	0.026 (0.061)	6.68E-01
SM C18:0	0.058 (0.056)	3.09E-01
SM C18:1	0.055 (0.065)	4.02E-01
SM C20:2	0.028 (0.069)	6.80E-01
SM C22:3	-0.013 (0.091)	8.89E-01
SM C24:0	0.038 (0.054)	4.76E-01
SM C24:1	0.027 (0.058)	6.44E-01
SM C26:0	-0.019 (0.064)	7.68E-01

SM C26:1	-0.015 (0.058)	7.91E-01
Ratios		
Acyl-carnitines		
(AC C16:0 + AC C18:0) / AC C0	0.294 (0.102)	4.27E-03
(AC C2:0 + AC C3:0) / AC C0	0.038 (0.049)	4.33E-01
AC C12:0 / AC C10:0	0.098 (0.055)	7.45E-02
AC C14:0 / AC C16:1	-0.010 (0.042)	8.05E-01
AC C2:0 / AC C0	0.051 (0.053)	3.42E-01
AC C3:0 / AC C4:0	0.006 (0.100)	9.56E-01
Amino acids		
Cit / Arg	0.214 (0.134)	1.11E-01
Cit / Orn	-0.004 (0.048)	9.37E-01
Fisher	-0.040 (0.038)	2.96E-01
Gly / PC ae C38:2	-0.001 (0.097)	9.89E-01
Orn / Arg	0.218 (0.136)	1.12E-01
Orn / Ser	-0.104 (0.052)	4.56E-02
Tyr / Phe	0.004 (0.031)	9.10E-01
Bile acids		
Conj. BA / unconj. BA	-0.150 (0.207)	4.70E-01
Prim. BA / sec. BA	-0.084 (0.202)	6.79E-01
Phospholipids		
LysoPC / PC	0.121 (0.053)	2.25E-02
MUFA PC / SFA PC	0.013 (0.029)	6.42E-01
PC aa C36:3 / PC aa C36:4	0.067 (0.060)	2.68E-01
PC aa C40:3 / PC aa C42:5	0.064 (0.053)	2.27E-01
PC ae C32:1 / PC ae C34:1	-0.017 (0.031)	5.84E-01
PC ae C38:1 / PC aa C28:1	-0.165 (0.083)	4.77E-02
PC ae C44:5 / PC ae C42:5	-0.035 (0.025)	1.67E-01
PUFA-PC / MUFA-PC	-0.016 (0.033)	6.25E-01
PUFA-PC / SFA-PC	-0.003 (0.027)	9.23E-01
SM (OH) C24:1 / SM C16:0	0.029 (0.043)	5.00E-01
Sums		
Acyl-carnitines		
AC C16:0 + AC C18:0	0.282 (0.106)	8.36E-03
AC C2:0 + AC C3:0	0.026 (0.069)	7.08E-01
Carnitines	0.103 (0.065)	1.16E-01
Even. carn	0.118 (0.069)	8.91E-02
Long. carn	0.310 (0.104)	3.05E-03
Medium. carn	0.249 (0.087)	4.45E-03
Odd. carn	0.007 (0.066)	9.18E-01
Amino acids		
AA	-0.003 (0.025)	8.96E-01
Arom. AA	-0.032 (0.029)	2.63E-01
BCAA	-0.062 (0.041)	1.29E-01

Ess. AA	-0.028 (0.027)	2.98E-01
Glucog. AA	0.039 (0.037)	2.99E-01
Noness. AA	0.017 (0.029)	5.62E-01
Bile acids		
Conj. BA	-0.007 (0.135)	9.59E-01
Prim. BA	-0.002 (0.131)	9.87E-01
Sec. BA	0.161 (0.193)	4.05E-01
Unconj. BA	0.204 (0.138)	1.41E-01
Phospholipids		
Long. PC	-0.101 (0.056)	7.35E-02
Long. PC aa	-0.111 (0.064)	8.31E-02
Long. PC ae	-0.069 (0.048)	1.52E-01
Long. SM	0.029 (0.054)	5.94E-01
Long. SM C	0.029 (0.055)	5.96E-01
Long. SM OH	0.047 (0.060)	4.37E-01
LysoPC	0.097 (0.053)	6.59E-02
Mono. PC	-0.009 (0.059)	8.81E-01
Mono. PC aa	-0.008 (0.059)	8.89E-01
Mono. PC ae	-0.016 (0.059)	7.81E-01
PC	-0.023 (0.048)	6.30E-01
PC aa	-0.022 (0.048)	6.44E-01
PC ae	-0.034 (0.052)	5.15E-01
Poly. PC	-0.025 (0.048)	6.04E-01
Poly. PC aa	-0.024 (0.048)	6.17E-01
Poly. PC ae	-0.034 (0.052)	5.13E-01
Sat. LysoPC	0.102 (0.057)	7.64E-02
Sat. PC	-0.022 (0.057)	6.98E-01
Sat. PC aa	-0.011 (0.060)	8.53E-01
Sat. PC ae	-0.069 (0.053)	1.97E-01
Saturmono. PC	-0.009 (0.059)	8.81E-01
Short. PC	0.008 (0.062)	8.97E-01
Short. PC aa	0.009 (0.063)	8.80E-01
Short. PC ae	-0.022 (0.063)	7.25E-01
SM	0.030 (0.054)	5.80E-01
SM (OH)	0.031 (0.061)	6.17E-01
SM C	0.030 (0.054)	5.74E-01
Unsat. lysoPC	0.084 (0.056)	1.35E-01
Very. lysoPC	-0.037 (0.026)	1.55E-01

Data are β-estimate (SE) of UCB metabolite concentrations, sums and ratios in offspring of obese mothers with high HbA1c at delivery (≥ 5.7 %) in reference to offspring of obese mothers with normal HbA1c at delivery (<5.7 %). Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear regression models adjusted for batch, GA, birth weight, mode of delivery, and performed in n = 106 offspring of obese mothers with high HbA1c at delivery and n = 181 offspring of obese mothers with normal HbA1c at delivery after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounders were excluded from the regression models. Bold font indicates p-values < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. BMI, body

mass index; GA; gestational age; GWG, gestational weight gain; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error; TG, triglyceride; UCB, umbilical cord blood.

Table S 9: Associations of UCB metabolites and the BMI z-score slope from age one to four years according to maternal BMI group

	Offspring of normal weight mothers		Offspring of obese mothers	
Metabolite	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
AC 2-M-C3:0	-0.010 (0.027)	7.13E-01	-0.011 (0.023)	6.33E-01
AC 2-M-C3:1	-0.013 (0.028)	6.45E-01	-0.016 (0.022)	4.55E-01
AC 2-M-C4:0	-0.012 (0.026)	6.54E-01	0.005 (0.023)	8.15E-01
AC 3-M-C4:0	0.020 (0.024)	4.16E-01	-0.063 (0.023)	**6.62E-03**
AC C0	0.002 (0.027)	9.39E-01	0.006 (0.023)	7.92E-01
AC C10:0	0.019 (0.030)	5.29E-01	-0.008 (0.021)	7.21E-01
AC C10:1	0.006 (0.030)	8.47E-01	0.001 (0.022)	9.51E-01
AC C11:0	0.049 (0.028)	8.29E-02	0.004 (0.022)	8.62E-01
AC C12:0	0.029 (0.028)	3.05E-01	-0.009 (0.022)	6.94E-01
AC C12:1	0.025 (0.027)	3.56E-01	-0.004 (0.022)	8.47E-01
AC C13:0	0.011 (0.028)	6.93E-01	-0.017 (0.022)	4.41E-01
AC C14:0	-0.008 (0.028)	7.78E-01	-0.008 (0.022)	7.18E-01
AC C14:1	0.009 (0.028)	7.42E-01	-0.007 (0.022)	7.50E-01
AC C15:0	-0.011 (0.027)	6.77E-01	-0.012 (0.022)	6.03E-01
AC C16:0	-0.014 (0.027)	6.03E-01	-0.019 (0.022)	3.78E-01
AC C16:1	0.002 (0.026)	9.36E-01	-0.012 (0.023)	5.90E-01
AC C17:0	0.007 (0.027)	8.00E-01	-0.031 (0.022)	1.56E-01
AC C18:0	-0.002 (0.029)	9.51E-01	-0.040 (0.021)	5.85E-02
AC C18:1	-0.002 (0.029)	9.49E-01	-0.024 (0.022)	2.72E-01
AC C18:2	-0.030 (0.027)	2.80E-01	-0.017 (0.022)	4.27E-01
AC C2:0	0.010 (0.025)	6.93E-01	-0.005 (0.023)	8.36E-01
AC C3:0	0.016 (0.025)	5.22E-01	-0.007 (0.023)	7.51E-01
AC C3-DC	0.020 (0.025)	4.15E-01	-0.014 (0.023)	5.40E-01
AC C3-M-DC	0.010 (0.028)	7.27E-01	-0.008 (0.022)	7.19E-01
AC C4:0	-0.005 (0.027)	8.51E-01	0.008 (0.022)	6.99E-01
AC C4:1	-0.002 (0.027)	9.28E-01	-0.013 (0.022)	5.60E-01
AC C4-DC	-0.007 (0.028)	8.07E-01	-0.027 (0.022)	2.15E-01
AC C4-OHa	-0.008 (0.024)	7.46E-01	0.026 (0.023)	2.71E-01
AC C4-OHb	-0.007 (0.030)	8.02E-01	-0.044 (0.021)	**3.70E-02**
AC C5:1	-0.011 (0.023)	6.39E-01	0.027 (0.024)	2.49E-01
AC C5-DC	0.028 (0.026)	2.92E-01	-0.025 (0.024)	2.89E-01
AC C5-M-DC	-0.024 (0.027)	3.70E-01	0.005 (0.023)	8.35E-01
AC C5-OH	0.009 (0.030)	7.77E-01	0.001 (0.022)	9.66E-01
AC C6:0	0.019 (0.027)	4.78E-01	0.002 (0.022)	9.31E-01
AC C6:1	-0.009 (0.031)	7.59E-01	-0.001 (0.021)	9.51E-01
AC C6-DC	0.012 (0.027)	6.68E-01	0.012 (0.022)	5.94E-01
AC C6-OHa	-0.013 (0.028)	6.35E-01	0.029 (0.022)	1.76E-01
AC C6-OHb	0.010 (0.025)	6.92E-01	0.016 (0.022)	4.75E-01
AC C7:0	0.010 (0.027)	7.11E-01	-0.013 (0.022)	5.58E-01
AC C7-DC	-0.007 (0.026)	7.88E-01	0.045 (0.022)	**4.45E-02**

AC C8:0	-0.003 (0.031)	9.21E-01	-0.025 (0.021)	2.44E-01
AC C8:1	-0.005 (0.029)	8.68E-01	0.011 (0.021)	6.22E-01
AC C8-OH	-0.011 (0.027)	6.87E-01	0.004 (0.022)	8.56E-01
AC C9:0	-0.012 (0.026)	6.38E-01	-0.001 (0.022)	9.63E-01
AC iso-C11:0	0.024 (0.025)	3.41E-01	-0.009 (0.022)	7.02E-01
AC iso-C13:0	0.021 (0.029)	4.66E-01	-0.019 (0.022)	3.83E-01
AC iso-C15:0	0.013 (0.028)	6.37E-01	-0.011 (0.022)	6.17E-01
AC iso-C17:0	-0.005 (0.026)	8.57E-01	-0.019 (0.022)	4.02E-01
AC iso-C9:0	0.005 (0.025)	8.48E-01	-0.008 (0.023)	7.27E-01
Amino acids				
1-M-His	-0.001 (0.026)	9.70E-01	0.001 (0.022)	9.68E-01
3-M-His	-0.002 (0.025)	9.32E-01	-0.012 (0.023)	5.97E-01
AAB	-0.003 (0.028)	9.11E-01	-0.006 (0.022)	7.75E-01
AADP	-0.026 (0.028)	3.48E-01	0.021 (0.021)	3.20E-01
Ala	-0.021 (0.026)	4.19E-01	-0.012 (0.023)	6.03E-01
Anserine	-0.035 (0.026)	1.80E-01	-0.032 (0.024)	1.92E-01
Arg	-0.016 (0.028)	5.76E-01	-0.004 (0.022)	8.69E-01
Asn	-0.042 (0.024)	8.86E-02	0.000 (0.024)	9.92E-01
Asp	-0.028 (0.026)	2.89E-01	-0.001 (0.023)	9.49E-01
BAIB	-0.033 (0.022)	1.41E-01	0.022 (0.024)	3.69E-01
Carnosine	-0.008 (0.029)	7.93E-01	-0.002 (0.022)	9.29E-01
Cit	-0.025 (0.026)	3.47E-01	0.001 (0.023)	9.50E-01
Cys-Cys	-0.001 (0.024)	9.62E-01	0.016 (0.024)	5.14E-01
Dimethylglycine	-0.020 (0.023)	3.76E-01	0.041 (0.024)	9.14E-02
GABA	-0.010 (0.030)	7.37E-01	0.005 (0.021)	8.12E-01
Gln	-0.023 (0.023)	3.29E-01	0.009 (0.023)	6.96E-01
Glu	-0.020 (0.029)	4.97E-01	0.002 (0.022)	9.12E-01
Gly	-0.023 (0.027)	3.83E-01	-0.032 (0.024)	1.77E-01
Hcys	-0.010 (0.028)	7.26E-01	-0.022 (0.023)	3.47E-01
His	-0.037 (0.028)	1.83E-01	-0.035 (0.023)	1.20E-01
Ile	0.003 (0.029)	9.16E-01	-0.031 (0.022)	1.47E-01
Leu	-0.004 (0.027)	8.80E-01	-0.051 (0.022)	**2.12E-02**
Lys	-0.048 (0.025)	5.63E-02	-0.003 (0.023)	8.83E-01
Met	-0.058 (0.025)	**2.40E-02**	0.009 (0.023)	7.10E-01
OH-Pro	-0.049 (0.030)	1.13E-01	0.024 (0.023)	2.91E-01
Orn	-0.058 (0.025)	**2.11E-02**	-0.016 (0.023)	5.00E-01
Phe	-0.039 (0.025)	1.30E-01	-0.018 (0.023)	4.16E-01
Pro	-0.003 (0.025)	9.14E-01	0.017 (0.023)	4.65E-01
Sarcosine	-0.016 (0.029)	5.83E-01	0.039 (0.022)	8.28E-02
Ser	-0.060 (0.025)	**1.81E-02**	-0.010 (0.023)	6.78E-01
Thr	-0.058 (0.022)	**8.67E-03**	-0.028 (0.024)	2.53E-01
Trp	0.051 (0.026)	5.39E-02	-0.018 (0.022)	4.26E-01
Tyr	-0.005 (0.031)	8.73E-01	-0.027 (0.021)	2.11E-01
Val	-0.017 (0.026)	4.97E-01	-0.044 (0.022)	5.18E-02

β-Ala	-0.002 (0.025)	9.20E-01	0.012 (0.023)	6.09E-01
Amino acid derivatives				
Betaine	-0.016 (0.029)	5.89E-01	0.044 (0.022)	**4.71E-02**
Choline	0.011 (0.025)	6.66E-01	-0.046 (0.023)	**4.93E-02**
Bile acids				
CA	0.059 (0.025)	**1.76E-02**	-0.011 (0.023)	6.38E-01
CDCA	0.056 (0.024)	**2.08E-02**	-0.017 (0.024)	4.83E-01
DCA	0.003 (0.026)	8.99E-01	-0.038 (0.023)	1.04E-01
GCA	0.051 (0.023)	**2.91E-02**	0.000 (0.025)	9.86E-01
GCDCA	0.021 (0.025)	3.97E-01	0.022 (0.023)	3.58E-01
GDCA	0.029 (0.025)	2.51E-01	-0.002 (0.023)	9.39E-01
GLCA	0.085 (0.043)	5.45E-02	0.050 (0.031)	1.15E-01
GUDCA	0.028 (0.027)	2.98E-01	-0.004 (0.023)	8.44E-01
LCA	0.012 (0.030)	6.84E-01	0.030 (0.031)	3.29E-01
TCA	0.040 (0.023)	8.60E-02	0.010 (0.025)	6.95E-01
TCDCA	0.004 (0.026)	8.87E-01	0.013 (0.023)	5.67E-01
TDCA	-0.001 (0.027)	9.61E-01	0.000 (0.023)	9.91E-01
TLCA	0.029 (0.026)	2.71E-01	0.013 (0.025)	6.18E-01
TLCA-S	0.019 (0.025)	4.45E-01	0.043 (0.024)	7.90E-02
TUDCA	-0.014 (0.029)	6.35E-01	0.021 (0.022)	3.42E-01
UDCA	0.033 (0.039)	3.95E-01	0.021 (0.029)	4.61E-01
Lipids				
HDL cholesterol	0.010 (0.027)	7.18E-01	-0.043 (0.022)	5.19E-02
LDL cholesterol	0.041 (0.026)	1.12E-01	-0.058 (0.022)	**9.07E-03**
TG	0.043 (0.026)	9.77E-02	-0.006 (0.023)	8.09E-01
Phospholipids				
LysoPC a C14:0	0.012 (0.029)	6.91E-01	-0.012 (0.021)	5.73E-01
LysoPC a C16:0	0.029 (0.027)	2.82E-01	-0.032 (0.022)	1.47E-01
LysoPC a C16:1	0.021 (0.029)	4.71E-01	-0.019 (0.022)	3.73E-01
LysoPC a C17:0	0.043 (0.024)	8.01E-02	-0.022 (0.023)	3.36E-01
LysoPC a C18:0	0.017 (0.026)	5.04E-01	-0.031 (0.022)	1.62E-01
LysoPC a C18:1	0.043 (0.030)	1.49E-01	-0.038 (0.022)	8.33E-02
LysoPC a C18:2	0.021 (0.028)	4.62E-01	-0.029 (0.022)	1.87E-01
LysoPC a C18:3	0.000 (0.026)	9.95E-01	-0.016 (0.022)	4.73E-01
LysoPC a C20:3	0.029 (0.026)	2.71E-01	-0.019 (0.022)	3.87E-01
LysoPC a C20:4	0.059 (0.029)	**4.78E-02**	-0.038 (0.022)	8.65E-02
LysoPC a C20:5	0.020 (0.024)	4.07E-01	0.003 (0.023)	8.90E-01
LysoPC a C22:5	0.013 (0.025)	5.96E-01	-0.044 (0.023)	5.73E-02
LysoPC a C22:6	0.006 (0.026)	8.22E-01	-0.026 (0.022)	2.43E-01
LysoPC a C24:0	-0.036 (0.028)	2.07E-01	0.019 (0.021)	3.70E-01
LysoPC a C26:0	0.003 (0.030)	9.34E-01	0.011 (0.021)	5.97E-01
LysoPC a C26:1	0.013 (0.028)	6.39E-01	0.007 (0.022)	7.35E-01
LysoPC a C28:0	-0.005 (0.026)	8.61E-01	-0.019 (0.022)	4.07E-01
LysoPC a C28:1	0.025 (0.025)	3.10E-01	-0.004 (0.023)	8.47E-01

PC aa C24:0	-0.003 (0.024)	8.99E-01	0.022 (0.024)	3.50E-01
PC aa C26:0	-0.010 (0.027)	7.25E-01	0.003 (0.023)	8.86E-01
PC aa C28:1	0.040 (0.025)	1.10E-01	-0.009 (0.023)	6.90E-01
PC aa C30:0	0.045 (0.027)	9.68E-02	-0.010 (0.022)	6.30E-01
PC aa C32:0	0.035 (0.026)	1.91E-01	-0.022 (0.022)	3.14E-01
PC aa C32:1	0.037 (0.028)	1.89E-01	-0.005 (0.022)	8.11E-01
PC aa C32:3	0.029 (0.027)	2.78E-01	-0.031 (0.022)	1.58E-01
PC aa C34:1	0.047 (0.027)	8.32E-02	-0.032 (0.022)	1.41E-01
PC aa C34:2	0.036 (0.028)	1.92E-01	-0.029 (0.021)	1.79E-01
PC aa C34:3	0.027 (0.030)	3.68E-01	-0.018 (0.021)	4.01E-01
PC aa C34:4	0.042 (0.031)	1.73E-01	-0.021 (0.021)	3.14E-01
PC aa C36:0	0.007 (0.028)	8.00E-01	-0.014 (0.023)	5.43E-01
PC aa C36:1	0.013 (0.027)	6.38E-01	-0.029 (0.022)	1.84E-01
PC aa C36:2	0.020 (0.028)	4.80E-01	-0.034 (0.022)	1.15E-01
PC aa C36:3	0.022 (0.025)	3.72E-01	-0.019 (0.022)	4.01E-01
PC aa C36:4	0.061 (0.027)	**2.61E-02**	-0.040 (0.021)	6.10E-02
PC aa C36:5	0.007 (0.023)	7.74E-01	-0.006 (0.023)	8.00E-01
PC aa C36:6	-0.003 (0.028)	9.12E-01	-0.015 (0.022)	4.78E-01
PC aa C38:0	0.007 (0.026)	8.02E-01	-0.027 (0.023)	2.33E-01
PC aa C38:1	0.015 (0.033)	6.49E-01	-0.029 (0.031)	3.53E-01
PC aa C38:3	0.003 (0.026)	8.95E-01	-0.019 (0.023)	3.90E-01
PC aa C38:4	0.042 (0.029)	1.42E-01	-0.045 (0.021)	**3.56E-02**
PC aa C38:5	0.013 (0.027)	6.29E-01	-0.029 (0.022)	1.87E-01
PC aa C38:6	-0.002 (0.027)	9.46E-01	-0.017 (0.022)	4.47E-01
PC aa C40:1	0.020 (0.029)	4.80E-01	-0.029 (0.022)	1.88E-01
PC aa C40:2	-0.006 (0.026)	8.12E-01	-0.042 (0.022)	5.79E-02
PC aa C40:3	0.005 (0.026)	8.58E-01	-0.036 (0.022)	1.08E-01
PC aa C40:4	-0.017 (0.029)	5.55E-01	-0.026 (0.022)	2.27E-01
PC aa C40:5	-0.016 (0.026)	5.52E-01	-0.031 (0.023)	1.74E-01
PC aa C40:6	-0.022 (0.028)	4.32E-01	-0.012 (0.022)	5.80E-01
PC aa C42:0	0.019 (0.029)	5.06E-01	-0.031 (0.022)	1.61E-01
PC aa C42:1	0.017 (0.027)	5.16E-01	-0.025 (0.022)	2.57E-01
PC aa C42:2	0.033 (0.030)	2.70E-01	-0.014 (0.021)	4.99E-01
PC aa C42:4	0.010 (0.028)	7.35E-01	-0.029 (0.021)	1.79E-01
PC aa C42:5	-0.007 (0.031)	8.29E-01	-0.016 (0.021)	4.59E-01
PC aa C42:6	-0.007 (0.030)	8.04E-01	-0.018 (0.022)	4.03E-01
PC ae C30:0	0.048 (0.025)	6.09E-02	-0.021 (0.023)	3.49E-01
PC ae C30:1	0.000 (0.000)	0.00E+00	-0.021 (0.091)	8.22E-01
PC ae C30:2	0.034 (0.027)	2.11E-01	-0.002 (0.022)	9.41E-01
PC ae C32:1	0.034 (0.025)	1.83E-01	-0.028 (0.022)	2.05E-01
PC ae C32:2	0.040 (0.026)	1.23E-01	-0.022 (0.022)	3.20E-01
PC ae C34:0	0.050 (0.026)	5.49E-02	-0.007 (0.023)	7.72E-01
PC ae C34:1	0.040 (0.027)	1.36E-01	-0.023 (0.022)	2.81E-01
PC ae C34:2	0.032 (0.027)	2.41E-01	-0.038 (0.021)	7.98E-02

PC ae C34:3	0.016 (0.029)	5.73E-01	-0.042 (0.022)	5.68E-02
PC ae C36:0	0.006 (0.027)	8.23E-01	-0.014 (0.022)	5.17E-01
PC ae C36:1	0.055 (0.026)	**3.64E-02**	-0.024 (0.022)	2.87E-01
PC ae C36:2	0.047 (0.028)	8.87E-02	-0.030 (0.022)	1.80E-01
PC ae C36:3	0.027 (0.027)	3.23E-01	-0.019 (0.022)	3.85E-01
PC ae C36:4	0.023 (0.028)	4.20E-01	-0.031 (0.022)	1.55E-01
PC ae C36:5	0.028 (0.026)	2.78E-01	-0.036 (0.022)	1.03E-01
PC ae C38:0	0.005 (0.026)	8.34E-01	-0.014 (0.022)	5.35E-01
PC ae C38:1	0.017 (0.029)	5.55E-01	-0.020 (0.022)	3.74E-01
PC ae C38:2	0.010 (0.026)	6.98E-01	-0.004 (0.024)	8.82E-01
PC ae C38:3	0.036 (0.026)	1.68E-01	-0.018 (0.023)	4.31E-01
PC ae C38:4	0.042 (0.028)	1.34E-01	-0.037 (0.022)	8.56E-02
PC ae C38:5	0.024 (0.027)	3.78E-01	-0.035 (0.022)	1.11E-01
PC ae C38:6	0.018 (0.026)	4.76E-01	-0.028 (0.022)	2.14E-01
PC ae C40:1	0.006 (0.028)	8.25E-01	-0.019 (0.021)	3.71E-01
PC ae C40:2	0.034 (0.027)	2.05E-01	-0.006 (0.022)	8.03E-01
PC ae C40:3	0.026 (0.026)	3.12E-01	-0.016 (0.023)	4.77E-01
PC ae C40:4	0.027 (0.028)	3.44E-01	-0.024 (0.022)	2.69E-01
PC ae C40:5	0.016 (0.028)	5.71E-01	-0.032 (0.022)	1.40E-01
PC ae C40:6	0.014 (0.028)	6.12E-01	-0.025 (0.023)	2.64E-01
PC ae C42:0	-0.002 (0.027)	9.52E-01	-0.012 (0.022)	5.90E-01
PC ae C42:1	0.004 (0.029)	8.95E-01	-0.008 (0.021)	6.97E-01
PC ae C42:2	0.007 (0.029)	8.19E-01	-0.017 (0.021)	4.31E-01
PC ae C42:3	0.005 (0.027)	8.64E-01	-0.022 (0.022)	3.12E-01
PC ae C42:4	0.016 (0.028)	5.77E-01	-0.036 (0.022)	9.82E-02
PC ae C42:5	0.027 (0.027)	3.17E-01	-0.036 (0.022)	9.99E-02
PC ae C44:3	-0.015 (0.031)	6.30E-01	-0.010 (0.021)	6.29E-01
PC ae C44:4	0.010 (0.029)	7.33E-01	-0.035 (0.021)	1.02E-01
PC ae C44:5	0.017 (0.028)	5.53E-01	-0.034 (0.022)	1.15E-01
PC ae C44:6	0.006 (0.028)	8.43E-01	-0.026 (0.022)	2.27E-01
SM (OH) C14:1	0.044 (0.026)	9.89E-02	-0.021 (0.023)	3.61E-01
SM (OH) C16:1	0.043 (0.027)	1.10E-01	-0.027 (0.023)	2.40E-01
SM (OH) C22:1	0.012 (0.024)	6.24E-01	-0.014 (0.024)	5.61E-01
SM (OH) C22:2	0.025 (0.025)	3.23E-01	-0.022 (0.023)	3.47E-01
SM (OH) C24:1	0.024 (0.025)	3.35E-01	-0.005 (0.024)	8.21E-01
SM C16:0	0.016 (0.027)	5.56E-01	-0.030 (0.022)	1.72E-01
SM C16:1	0.012 (0.027)	6.73E-01	-0.031 (0.021)	1.52E-01
SM C18:0	0.018 (0.027)	5.07E-01	-0.033 (0.022)	1.26E-01
SM C18:1	0.012 (0.027)	6.54E-01	-0.039 (0.021)	6.65E-02
SM C20:2	0.030 (0.027)	2.68E-01	-0.015 (0.022)	5.19E-01
SM C22:3	0.020 (0.025)	4.09E-01	-0.034 (0.023)	1.47E-01
SM C24:0	-0.003 (0.027)	9.00E-01	-0.021 (0.023)	3.59E-01
SM C24:1	0.022 (0.027)	4.07E-01	-0.034 (0.022)	1.19E-01
SM C26:0	0.034 (0.028)	2.22E-01	-0.006 (0.023)	7.91E-01

SM C26:1	0.028 (0.028)	3.25E-01	-0.017 (0.023)	4.51E-01
Ratios	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
(AC C16:0 + AC C18:0) / AC C0	-0.013 (0.027)	6.37E-01	-0.024 (0.022)	2.74E-01
(AC C2:0 + AC C3:0) / AC C0	0.009 (0.025)	7.35E-01	-0.014 (0.023)	5.28E-01
AC C12:0 / AC C10:0	0.017 (0.024)	4.85E-01	-0.021 (0.024)	3.76E-01
AC C14:0 / AC C16:1	-0.020 (0.024)	3.89E-01	0.013 (0.024)	5.69E-01
AC C2:0 / AC C0	0.013 (0.026)	6.14E-01	-0.011 (0.022)	6.16E-01
AC C3:0 / AC C4:0	0.003 (0.026)	9.10E-01	-0.021 (0.023)	3.58E-01
Amino acids				
Cit / Arg	0.003 (0.028)	9.10E-01	0.004 (0.022)	8.58E-01
Cit / Orn	0.022 (0.026)	3.96E-01	0.013 (0.023)	5.56E-01
Fisher	-0.008 (0.028)	7.74E-01	-0.028 (0.022)	1.99E-01
Gly / PC ae C38:2	-0.020 (0.026)	4.37E-01	-0.010 (0.023)	6.72E-01
Orn / Arg	-0.006 (0.027)	8.10E-01	-0.001 (0.022)	9.63E-01
Orn / Ser	0.022 (0.026)	3.96E-01	0.000 (0.022)	9.90E-01
Tyr / Phe	0.041 (0.028)	1.39E-01	-0.016 (0.022)	4.64E-01
Bile acids				
Conj. BA / unconj. BA	0.017 (0.026)	5.29E-01	0.017 (0.022)	4.47E-01
Prim. BA / sec. BA	0.007 (0.024)	7.63E-01	0.017 (0.023)	4.77E-01
Phospholipids				
LysoPC / PC	0.002 (0.029)	9.43E-01	0.000 (0.022)	9.89E-01
MUFA PC / SFA PC	0.013 (0.024)	5.75E-01	-0.022 (0.025)	3.70E-01
PC aa C36:3 / PC aa C36:4	-0.027 (0.026)	3.03E-01	0.023 (0.023)	3.13E-01
PC aa C40:3 / PC aa C42:5	0.012 (0.029)	6.74E-01	-0.013 (0.021)	5.33E-01
PC ae C32:1 / PC ae C34:1	0.001 (0.023)	9.72E-01	-0.015 (0.024)	5.31E-01
PC ae C38:1 / PC aa C28:1	-0.013 (0.026)	6.30E-01	-0.016 (0.023)	4.86E-01
PC ae C44:5 / PC ae C42:5	-0.022 (0.029)	4.45E-01	-0.015 (0.022)	4.79E-01
PUFA-PC / MUFA-PC	-0.026 (0.027)	3.47E-01	-0.003 (0.023)	8.98E-01
PUFA-PC / SFA-PC	-0.010 (0.025)	7.01E-01	-0.022 (0.023)	3.20E-01
SM (OH) C24:1 / SM C16:0	0.017 (0.024)	4.81E-01	0.028 (0.024)	2.33E-01
Sums	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
AC C16:0 + AC C18:0	-0.014 (0.028)	6.17E-01	-0.020 (0.022)	3.48E-01
AC C2:0 + AC C3:0	0.007 (0.025)	7.82E-01	-0.006 (0.023)	7.82E-01
Carnitines	0.007 (0.026)	7.92E-01	-0.018 (0.022)	4.13E-01
Even. carn	0.007 (0.027)	8.01E-01	-0.013 (0.022)	5.65E-01
Long. carn	-0.013 (0.028)	6.53E-01	-0.020 (0.022)	3.49E-01
Medium. carn	0.009 (0.029)	7.61E-01	-0.007 (0.022)	7.42E-01
Odd. carn	0.018 (0.027)	4.95E-01	-0.012 (0.022)	5.94E-01
Amino acids				
AA	-0.045 (0.023)	5.72E-02	-0.024 (0.024)	3.11E-01
Arom. AA	-0.005 (0.028)	8.61E-01	-0.028 (0.022)	1.98E-01
BCAA	-0.010 (0.026)	7.10E-01	-0.048 (0.022)	**3.16E-02**

Ess. AA	-0.048 (0.023)	**3.55E-02**	-0.044 (0.024)	6.35E-02
Glucog. AA	-0.036 (0.026)	1.76E-01	-0.019 (0.024)	4.16E-01
Noness. AA	-0.036 (0.025)	1.46E-01	-0.009 (0.024)	7.19E-01
Bile acids				
Conj. BA	0.035 (0.024)	1.46E-01	0.014 (0.024)	5.55E-01
Prim. BA	0.036 (0.023)	1.33E-01	0.014 (0.025)	5.62E-01
Sec. BA	0.003 (0.025)	9.02E-01	-0.008 (0.022)	7.10E-01
Unconj. BA	0.050 (0.024)	**3.77E-02**	-0.021 (0.023)	3.66E-01
Phospholipids				
Long. PC	-0.015 (0.029)	6.04E-01	-0.020 (0.022)	3.76E-01
Long. PC aa	-0.023 (0.029)	4.19E-01	-0.017 (0.022)	4.54E-01
Long. PC ae	0.019 (0.028)	4.92E-01	-0.027 (0.022)	2.17E-01
Long. SM	0.015 (0.027)	5.75E-01	-0.031 (0.022)	1.66E-01
Long. SM C	0.015 (0.027)	5.87E-01	-0.031 (0.022)	1.62E-01
Long. SM OH	0.024 (0.025)	3.35E-01	-0.005 (0.024)	8.21E-01
LysoPC	0.033 (0.027)	2.22E-01	-0.035 (0.022)	1.07E-01
Mono. PC	0.043 (0.027)	1.14E-01	-0.030 (0.022)	1.72E-01
Mono. PC aa	0.042 (0.027)	1.20E-01	-0.030 (0.022)	1.70E-01
Mono. PC ae	0.045 (0.027)	1.01E-01	-0.025 (0.022)	2.45E-01
PC	0.033 (0.026)	2.14E-01	-0.035 (0.022)	1.09E-01
PC aa	0.033 (0.026)	2.16E-01	-0.035 (0.022)	1.09E-01
PC ae	0.032 (0.027)	2.38E-01	-0.032 (0.022)	1.44E-01
Poly. PC	0.030 (0.026)	2.55E-01	-0.035 (0.022)	1.05E-01
Poly. PC aa	0.030 (0.026)	2.58E-01	-0.035 (0.022)	1.07E-01
Poly. PC ae	0.030 (0.027)	2.70E-01	-0.034 (0.022)	1.24E-01
Sat. LysoPC	0.027 (0.026)	3.06E-01	-0.032 (0.022)	1.49E-01
Sat. PC	0.031 (0.026)	2.28E-01	-0.021 (0.022)	3.32E-01
Sat. PC aa	0.031 (0.026)	2.34E-01	-0.022 (0.022)	3.06E-01
Sat. PC ae	0.024 (0.027)	3.78E-01	-0.012 (0.022)	5.87E-01
Saturmono. PC	0.043 (0.027)	1.14E-01	-0.030 (0.022)	1.72E-01
Short. PC	0.044 (0.028)	1.14E-01	-0.030 (0.021)	1.65E-01
Short. PC aa	0.044 (0.028)	1.15E-01	-0.030 (0.021)	1.67E-01
Short. PC ae	0.038 (0.026)	1.43E-01	-0.030 (0.022)	1.72E-01
SM	0.018 (0.027)	5.08E-01	-0.033 (0.022)	1.34E-01
SM (OH)	0.026 (0.025)	2.95E-01	-0.021 (0.023)	3.75E-01
SM C	0.016 (0.027)	5.45E-01	-0.033 (0.022)	1.27E-01
Unsat. lysoPC	0.038 (0.029)	1.91E-01	-0.033 (0.022)	1.25E-01
Very. lysoPC	0.003 (0.028)	9.00E-01	-0.015 (0.022)	5.13E-01

Data are β-estimate (SE) of the BMI z-score slopes from age one to four years per metabolite, sums and ratios based on offspring of normal weight and offspring of obese (preconception BMI ≥ 29.5 kg/m^2) mothers. Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear regression models adjusted for GWG, smoking during pregnancy, breastfeeding, and SES (Model 1) performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the regression models. Bold font indicates p-value < 0.05. A full list of metabolite abbreviations is provided

in Table S 1, of sums and ratios in Table S 2. BMI, body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status; TG, triglyceride; UCB, umbilical cord blood.

Table S 10: Sensitivity analysis of significant associations (p < 0.05) between UCB metabolites and the BMI z-score slope from age one to four years using multiple linear regression models

	Model 1a		Model 2				Model 3			
	All offspring		Offspring of normal weight mothers		Offspring of obese mothers		Offspring of normal weight mothers		Offspring of obese mothers	
Metabolites	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Leu	-0.037 (0.017)	**3.55E-02**	0.003 (0.027)	9.03E-01	-0.036 (0.022)	1.07E-01	0.006 (0.027)	8.29E-01	-0.039 (0.022)	8.33E-02
Met	-0.010 (0.018)	5.79E-01	-0.048 (0.026)	6.69E-02	0.014 (0.023)	5.45E-01	-0.048 (0.026)	7.18E-02	0.013 (0.023)	5.68E-01
Thr	-0.039 (0.018)	**2.66E-02**	-0.050 (0.023)	**3.32E-02**	-0.014 (0.023)	5.60E-01	-0.050 (0.023)	**3.36E-02**	-0.015 (0.024)	5.33E-01
Ser	-0.025 (0.018)	1.69E-01	-0.050 (0.026)	6.34E-02	0.002 (0.023)	9.36E-01	-0.051 (0.027)	5.49E-02	0.003 (0.023)	8.92E-01
Orn	-0.028 (0.018)	1.17E-01	-0.052 (0.025)	**3.50E-02**	-0.013 (0.023)	5.69E-01	-0.050 (0.025)	**4.34E-02**	-0.015 (0.023)	5.09E-01
Betaine	0.028 (0.018)	1.14E-01	-0.007 (0.028)	7.98E-01	0.032 (0.022)	1.56E-01	-0.003 (0.028)	9.17E-01	0.033 (0.022)	1.41E-01
Choline	-0.030 (0.018)	9.77E-02	0.016 (0.025)	5.32E-01	-0.039 (0.023)	9.28E-02	0.012 (0.025)	6.29E-01	-0.037 (0.023)	1.08E-01
AC 3-M-C4:0	-0.037 (0.018)	**3.41E-02**	0.018 (0.024)	4.65E-01	-0.058 (0.023)	**1.08E-02**	0.020 (0.024)	4.21E-01	-0.058 (0.023)	**1.07E-02**
AC C4:1	-0.035 (0.017)	**4.68E-02**	-0.013 (0.027)	6.40E-01	-0.063 (0.022)	**3.64E-03**	-0.015 (0.027)	5.80E-01	-0.058 (0.022)	7.61E-03
AC C7:0	0.032 (0.018)	7.12E-02	0.003 (0.025)	9.17E-01	0.040 (0.023)	7.58E-02	0.006 (0.025)	8.06E-01	0.041 (0.023)	7.45E-02
CA	0.011 (0.017)	5.44E-01	0.058 (0.024)	**1.76E-02**	0.000 (0.023)	9.83E-01	0.059 (0.024)	**1.61E-02**	0.000 (0.023)	9.98E-01
GCA	0.022 (0.018)	2.23E-01	0.047 (0.023)	**4.15E-02**	0.008 (0.025)	7.41E-01	0.051 (0.023)	**2.67E-02**	0.010 (0.025)	7.01E-01
CDCA	0.005 (0.018)	7.58E-01	0.065 (0.023)	**5.47E-03**	-0.019 (0.024)	4.20E-01	0.059 (0.023)	**1.09E-02**	-0.017 (0.024)	4.68E-01
LysoPC a C20:4	-0.018 (0.018)	3.03E-01	0.055 (0.027)	**4.31E-02**	-0.040 (0.022)	6.97E-02	0.055 (0.027)	**4.45E-02**	-0.038 (0.022)	8.47E-02
PC aa C36:4	-0.017 (0.017)	3.34E-01	0.064 (0.026)	**1.77E-02**	-0.031 (0.022)	1.48E-01	0.060 (0.027)	**2.62E-02**	-0.034 (0.022)	1.20E-01
PC aa C38:4	-0.026 (0.017)	1.40E-01	0.043 (0.028)	1.30E-01	-0.032 (0.021)	1.35E-01	0.041 (0.029)	1.55E-01	-0.035 (0.021)	9.92E-02
PC ae C36:1	-0.002 (0.018)	9.08E-01	0.052 (0.024)	**3.60E-02**	-0.005 (0.022)	8.08E-01	0.051 (0.025)	**4.16E-02**	-0.009 (0.022)	6.89E-01
LDL cholesterol	-0.030 (0.017)	8.87E-02	0.034 (0.026)	1.84E-01	-0.052 (0.022)	**1.84E-02**	0.035 (0.026)	1.76E-01	-0.053 (0.022)	**1.59E-02**
Ratios and Sums										
BCAAs	-0.036 (0.017)	**4.01E-02**	0.004 (0.026)	8.73E-01	-0.032 (0.023)	1.58E-01	0.005 (0.026)	8.60E-01	-0.035 (0.023)	1.26E-01
Ess. AAs	-0.045 (0.018)	**9.76E-03**	-0.038 (0.024)	1.12E-01	-0.032 (0.023)	1.77E-01	-0.039 (0.024)	1.01E-01	-0.033 (0.023)	1.56E-01
unconj. BAs	0.001 (0.017)	9.50E-01	0.056 (0.023)	**1.93E-02**	-0.013 (0.023)	5.68E-01	0.056 (0.023)	**1.84E-02**	-0.011 (0.023)	6.39E-01

Data are β-estimate (SE) of the BMI z-score slope from age one to four years per metabolite, sums and ratios of significant findings based on offspring of normal weight and those of obese (preconception BMI ≥ 29.5 kg/m^2) mothers analyzed in different linear regression models, modified from Model 1, performed in n = 111

offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the regression models. Concentrations of LDL cholesterol is provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Bold font indicates p-value < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2.

Model 1: BMI z-score slope ~ metabolite (scaled) + age (years) + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + SES; Random effect: 1|ID

Model 1a: BMI z-score slope ~ metabolite (scaled) + age (years) + maternal preconception BMI (normal weight and obese, ≥29.5 kg/m2) + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + SES; Random effect: 1|ID

Model 2: BMI z-score slope ~ metabolite (scaled) + age (years) + maternal HbA1c (%) at delivery + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + parental SES at birth; Random effect: 1|ID

Model 3: BMI z-score slope~ metabolite (residuals) + age (years) + GWG + smoking anytime during pregnancy breastfeeding (predominant ≥1 month) + parental SES at birth; Random effect: 1|ID

Metabolite (residuals) presents the value after correcting the metabolite log2-transformed concentrations for potential confounding factors used to investigate association between UCB metabolites and maternal obesity; Metabolite (scaled) presents the log2-transformed metabolite concentrations scaled to a mean of 0 and SD of 1; BMI, body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status; TG, triglyceride; UCB, umbilical cord blood.

Table S 11: Significant associations (p < 0.05) of UCB metabolites and the BMI z-score slope from age one to four years according to maternal BMI group and offspring sex

	Offspring of normal weight mothers						Offspring of obese mothers					
	All		Male		Female		All		Male		Female	
Metabolites	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Leu	-0.004 (0.027)	8.80E-01	-0.029 (0.039)	4.60E-01	0.007 (0.036)	8.42E-01	-0.051 (0.022)	**2.12E-02**	-0.051 (0.031)	1.04E-01	-0.052 (0.033)	1.19E-01
Met	-0.058 (0.025)	**2.40E-02**	-0.068 (0.038)	8.40E-02	-0.059 (0.033)	8.53E-02	0.009 (0.023)	7.10E-01	-0.001 (0.034)	9.86E-01	0.013 (0.032)	6.83E-01
Thr	-0.058 (0.022)	**8.67E-03**	-0.057 (0.041)	1.72E-01	-0.049 (0.024)	**4.78E-02**	-0.028 (0.024)	2.53E-01	-0.071 (0.039)	6.68E-02	0.000 (0.030)	9.94E-01
Ser	-0.060 (0.025)	**1.81E-02**	-0.074 (0.040)	6.96E-02	-0.043 (0.031)	1.75E-01	-0.010 (0.023)	6.78E-01	-0.044 (0.035)	2.10E-01	0.016 (0.031)	6.08E-01
Orn	-0.058 (0.025)	**2.11E-02**	-0.051 (0.043)	2.39E-01	-0.064 (0.029)	**3.31E-02**	-0.016 (0.023)	5.00E-01	-0.021 (0.035)	5.43E-01	-0.022 (0.031)	4.82E-01
Betaine	-0.016 (0.029)	5.89E-01	-0.035 (0.041)	3.97E-01	0.006 (0.043)	8.91E-01	0.044 (0.022)	**4.71E-02**	0.035 (0.033)	2.91E-01	0.047 (0.033)	1.61E-01
Choline	0.011 (0.025)	6.66E-01	0.033 (0.044)	4.55E-01	-0.018 (0.030)	5.35E-01	-0.046 (0.023)	**4.93E-02**	-0.054 (0.035)	1.24E-01	-0.043 (0.032)	1.79E-01
AC 3-M-C4:0	0.020 (0.024)	4.16E-01	-0.023 (0.046)	6.13E-01	0.024 (0.027)	3.87E-01	-0.063 (0.023)	**6.62E-03**	-0.095 (0.037)	**1.09E-02**	-0.033 (0.030)	2.77E-01
AC C4:1	-0.007 (0.030)	8.02E-01	-0.091 (0.054)	1.02E-01	0.080 (0.033)	**1.84E-02**	-0.044 (0.021)	**3.70E-02**	-0.099 (0.032)	**2.36E-03**	0.008 (0.029)	7.92E-01
AC C7:0	-0.007 (0.026)	7.88E-01	-0.038 (0.046)	4.06E-01	0.029 (0.030)	3.35E-01	0.045 (0.022)	**4.45E-02**	0.071 (0.035)	**4.44E-02**	0.008 (0.031)	8.09E-01
CA	0.059 (0.025)	**1.76E-02**	0.064 (0.043)	1.48E-01	0.053 (0.028)	6.63E-02	-0.011 (0.023)	6.38E-01	0.001 (0.034)	9.75E-01	-0.014 (0.031)	6.55E-01
GCA	0.051 (0.023)	**2.91E-02**	0.019 (0.040)	6.37E-01	0.074 (0.026)	**5.42E-03**	0.000 (0.025)	9.86E-01	0.004 (0.040)	9.25E-01	0.002 (0.032)	9.53E-01
CDCA	0.056 (0.024)	**2.08E-02**	0.077 (0.041)	6.53E-02	0.037 (0.027)	1.78E-01	-0.017 (0.024)	4.83E-01	-0.029 (0.037)	4.29E-01	0.000 (0.030)	9.98E-01
LysoPC a C20:4	0.059 (0.029)	**4.78E-02**	0.053 (0.050)	2.94E-01	0.042 (0.037)	2.65E-01	-0.038 (0.022)	8.65E-02	-0.027 (0.032)	3.98E-01	-0.053 (0.032)	9.48E-02
PC aa C36:4	0.061 (0.027)	**2.61E-02**	0.076 (0.045)	9.57E-02	0.020 (0.037)	5.89E-01	-0.040 (0.021)	6.10E-02	-0.060 (0.030)	**4.61E-02**	-0.002 (0.033)	9.54E-01
PC aa C38:4	0.042 (0.029)	1.42E-01	0.040 (0.046)	3.91E-01	0.002 (0.040)	9.56E-01	-0.045 (0.021)	**3.56E-02**	-0.066 (0.029)	**2.61E-02**	-0.003 (0.033)	9.24E-01
PC ae C36:1	0.055 (0.026)	**3.64E-02**	0.077 (0.042)	7.65E-02	0.013 (0.032)	6.79E-01	-0.031 (0.022)	1.55E-01	-0.048 (0.033)	1.42E-01	0.009 (0.032)	7.69E-01
LDL cholesterol	0.041 (0.026)	1.12E-01	0.015 (0.045)	7.41E-01	0.034 (0.034)	3.17E-01	-0.058 (0.022)	**9.07E-03**	-0.094 (0.032)	**4.18E-03**	-0.015 (0.031)	6.14E-01
Ratios and Sums												
BCAAs	-0.010 (0.026)	7.10E-01	-0.034 (0.038)	3.82E-01	-0.002 (0.035)	9.46E-01	-0.048 (0.022)	**3.16E-02**	-0.044 (0.032)	1.69E-01	-0.060 (0.032)	6.60E-02
Ess. AAs	-0.048 (0.023)	**3.55E-02**	-0.060 (0.035)	9.64E-02	-0.049 (0.028)	9.04E-02	-0.044 (0.024)	**6.35E-02**	-0.047 (0.034)	1.63E-01	-0.049 (0.034)	1.45E-01
unconj. BAs	0.050 (0.024)	**3.77E-02**	0.041 (0.041)	3.25E-01	0.048 (0.028)	8.63E-02	-0.021 (0.023)	3.66E-01	-0.025 (0.036)	4.84E-01	-0.013 (0.030)	6.71E-01

Data are β-estimate (SE) of the BMI z-score slope from age one to four years per metabolite, sums and ratios of significant findings in offspring of normal weight and those of obese (preconception BMI ≥ 29.5 kg/m^2) mothers and those stratified by offspring sex. Concentrations of LDL cholesterol is provided in mg/dL. Bile

acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear regression models adjusted for GWG, smoking during pregnancy, breastfeeding, and SES (Model 1) performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the regression models. The analysis was repeated after dataset was stratified by offspring sex. Bold font indicates p-value < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. BMI, body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status UCB, umbilical cord blood.

Table S 12: Associations of UCB metabolites and BMI z-scores at ages one, two, three, and four years according to maternal BMI group

	Offspring of normal weight mothers		Offspring of obese mothers	
Metabolite	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
AC 2-M-C3:0	0.744 (-0.005)	9.56E-01	-0.036 (0.056)	5.17E-01
AC 2-M-C3:1	0.444 (-0.014)	8.75E-01	-0.051 (0.054)	3.42E-01
AC 2-M-C4:0	0.367 (-0.173)	**4.19E-02**	-0.013 (0.056)	8.15E-01
AC 3-M-C4:0	0.132 (-0.107)	1.67E-01	-0.078 (0.057)	1.72E-01
AC C0	0.805 (0.019)	8.25E-01	-0.026 (0.055)	6.45E-01
AC C10:0	0.320 (-0.063)	5.16E-01	-0.035 (0.052)	4.97E-01
AC C10:1	0.520 (0.010)	9.16E-01	0.042 (0.053)	4.29E-01
AC C11:0	0.183 (-0.039)	6.70E-01	-0.063 (0.053)	2.37E-01
AC C12:0	0.422 (0.006)	9.48E-01	-0.037 (0.054)	4.93E-01
AC C12:1	0.942 (-0.010)	9.11E-01	0.009 (0.055)	8.74E-01
AC C13:0	0.200 (-0.097)	2.82E-01	-0.032 (0.054)	5.53E-01
AC C14:0	0.965 (0.065)	4.73E-01	-0.009 (0.054)	8.73E-01
AC C14:1	0.713 (0.022)	8.08E-01	0.023 (0.053)	6.70E-01
AC C15:0	0.971 (0.041)	6.33E-01	-0.005 (0.055)	9.26E-01
AC C16:0	0.543 (0.023)	7.98E-01	0.034 (0.053)	5.28E-01
AC C16:1	1.000 (0.036)	6.77E-01	-0.007 (0.055)	8.96E-01
AC C17:0	0.968 (-0.012)	8.87E-01	0.014 (0.054)	7.92E-01
AC C18:0	0.772 (0.031)	7.45E-01	0.012 (0.052)	8.17E-01
AC C18:1	0.881 (0.024)	7.95E-01	0.003 (0.053)	9.56E-01
AC C18:2	0.589 (0.073)	4.12E-01	0.015 (0.054)	7.83E-01
AC C2:0	0.320 (0.039)	6.26E-01	-0.090 (0.055)	1.07E-01
AC C3:0	0.627 (0.017)	8.58E-01	-0.046 (0.054)	3.90E-01
AC C3-DC	0.594 (0.011)	8.91E-01	-0.048 (0.057)	3.96E-01
AC C3-M-DC	0.370 (-0.048)	5.50E-01	-0.027 (0.055)	6.30E-01
AC C4:0	0.936 (0.071)	3.59E-01	-0.045 (0.057)	4.25E-01
AC C4:1	0.271 (-0.019)	8.46E-01	-0.070 (0.052)	1.83E-01
AC C4-DC	0.915 (0.070)	4.23E-01	-0.029 (0.053)	5.90E-01
AC C4-OHa	0.273 (-0.003)	9.74E-01	-0.073 (0.054)	1.72E-01
AC C4-OHb	0.356 (0.034)	7.06E-01	-0.078 (0.054)	1.48E-01
AC C5:1	0.110 (-0.131)	1.79E-01	-0.083 (0.054)	1.23E-01
AC C5-DC	0.707 (0.048)	5.23E-01	-0.068 (0.058)	2.41E-01
AC C5-M-DC	0.202 (0.027)	7.56E-01	-0.059 (0.058)	3.09E-01
AC C5-OH	0.969 (0.008)	9.26E-01	-0.025 (0.056)	6.61E-01
AC C6:0	0.122 (-0.060)	5.12E-01	-0.080 (0.053)	1.30E-01
AC C6:1	0.548 (-0.046)	5.76E-01	-0.019 (0.055)	7.36E-01
AC C6-DC	0.214 (-0.170)	5.24E-02	-0.038 (0.054)	4.82E-01
AC C6-OHa	0.154 (-0.097)	3.35E-01	-0.076 (0.052)	1.47E-01
AC C6-OHb	0.085 (-0.080)	3.59E-01	-0.096 (0.054)	7.94E-02
AC C7:0	0.924 (0.069)	4.19E-01	-0.017 (0.055)	7.60E-01

AC C7-DC	0.306 (-0.026)	7.66E-01	-0.052 (0.053)	3.24E-01
AC C8:0	0.633 (-0.042)	6.55E-01	-0.011 (0.053)	8.32E-01
AC C8:1	0.694 (-0.109)	2.19E-01	0.001 (0.055)	9.82E-01
AC C8-OH	0.131 (-0.064)	5.22E-01	-0.073 (0.052)	1.66E-01
AC C9:0	0.194 (0.065)	4.45E-01	0.068 (0.055)	2.19E-01
AC iso-C11:0	0.672 (-0.011)	8.94E-01	0.040 (0.055)	4.71E-01
AC iso-C13:0	0.567 (-0.001)	9.88E-01	0.050 (0.054)	3.57E-01
AC iso-C15:0	0.725 (0.040)	6.63E-01	0.021 (0.054)	6.94E-01
AC iso-C17:0	0.748 (0.075)	3.69E-01	-0.041 (0.054)	4.54E-01
AC iso-C9:0	0.589 (0.019)	8.12E-01	0.037 (0.057)	5.25E-01
Amino acids				
1-M-His	0.236 (0.016)	8.53E-01	-0.085 (0.054)	1.18E-01
3-M-His	0.838 (0.023)	7.74E-01	-0.041 (0.057)	4.71E-01
AAB	0.080 (0.009)	9.24E-01	-0.113 (0.053)	**3.35E-02**
AADP	0.770 (0.032)	7.21E-01	-0.033 (0.053)	5.27E-01
Ala	0.771 (0.050)	5.59E-01	-0.026 (0.058)	6.46E-01
Anserine	0.139 (-0.015)	8.49E-01	-0.116 (0.062)	6.32E-02
Arg	0.117 (-0.071)	4.35E-01	-0.067 (0.054)	2.17E-01
Asn	0.840 (0.133)	9.40E-02	-0.054 (0.058)	3.54E-01
Asp	0.704 (0.052)	5.34E-01	0.031 (0.055)	5.72E-01
BAIB	0.034 (0.072)	3.35E-01	0.118 (0.058)	**4.37E-02**
Carnosine	0.318 (0.005)	9.54E-01	0.048 (0.054)	3.78E-01
Cit	0.051 (0.086)	3.08E-01	0.098 (0.056)	7.74E-02
Cys-Cys	0.218 (-0.005)	9.51E-01	-0.077 (0.058)	1.89E-01
Dimethylglycine	0.492 (0.120)	1.02E-01	-0.023 (0.060)	7.08E-01
GABA	0.543 (0.137)	1.54E-01	0.015 (0.054)	7.84E-01
Gln	0.947 (0.024)	7.49E-01	-0.001 (0.057)	9.81E-01
Glu	0.494 (0.045)	6.31E-01	0.043 (0.053)	4.13E-01
Gly	0.946 (0.065)	4.56E-01	0.004 (0.059)	9.42E-01
Hcys	0.823 (-0.031)	7.36E-01	0.021 (0.056)	7.03E-01
His	0.559 (0.078)	3.93E-01	0.035 (0.056)	5.28E-01
Ile	0.662 (-0.065)	4.87E-01	-0.007 (0.053)	8.91E-01
Leu	0.865 (0.024)	7.86E-01	-0.020 (0.055)	7.17E-01
Lys	0.094 (0.079)	3.40E-01	0.078 (0.056)	1.65E-01
Met	0.538 (0.117)	1.65E-01	0.009 (0.056)	8.79E-01
OH-Pro	0.038 (0.154)	1.21E-01	0.059 (0.055)	2.85E-01
Orn	0.050 (0.156)	5.51E-02	0.084 (0.056)	1.36E-01
Phe	0.940 (-0.023)	7.86E-01	0.011 (0.055)	8.36E-01
Pro	0.077 (0.173)	**3.36E-02**	0.060 (0.056)	2.91E-01
Sarcosine	0.955 (0.116)	2.14E-01	-0.026 (0.055)	6.35E-01
Ser	0.789 (0.014)	8.69E-01	-0.010 (0.057)	8.64E-01
Thr	0.108 (0.112)	1.23E-01	0.050 (0.059)	3.99E-01
Trp	0.253 (-0.065)	4.50E-01	-0.045 (0.054)	4.11E-01
Tyr	0.783 (-0.009)	9.31E-01	-0.019 (0.052)	7.12E-01

Val	0.931 (0.037)	6.58E-01	-0.021 (0.056)	6.99E-01
β-Ala	0.419 (-0.021)	7.91E-01	0.072 (0.058)	2.14E-01
Amino acid derivatives				
Betaine	0.585 (-0.024)	7.96E-01	-0.036 (0.054)	5.05E-01
Choline	0.301 (-0.056)	4.86E-01	-0.045 (0.057)	4.25E-01
Bile acids				
CA	0.170 (-0.157)	5.61E-02	-0.019 (0.056)	7.34E-01
CDCA	0.815 (-0.103)	1.91E-01	0.018 (0.058)	7.51E-01
DCA	0.469 (-0.216)	**1.03E-02**	0.025 (0.058)	6.61E-01
GCA	0.001 (-0.124)	1.01E-01	-0.162 (0.061)	**8.42E-03**
GCDCA	0.590 (-0.078)	3.40E-01	-0.003 (0.058)	9.60E-01
GDCA	0.044 (-0.271)	**7.75E-04**	-0.011 (0.056)	8.50E-01
GLCA	0.701 (-0.107)	4.90E-01	0.069 (0.079)	3.85E-01
GUDCA	0.004 (-0.193)	**3.14E-02**	-0.107 (0.055)	5.05E-02
LCA	0.815 (0.056)	5.61E-01	-0.007 (0.080)	9.33E-01
TCA	0.002 (-0.081)	2.85E-01	-0.166 (0.060)	**6.12E-03**
TCDCA	0.696 (-0.030)	7.20E-01	-0.014 (0.057)	8.04E-01
TDCA	0.227 (-0.187)	**2.44E-02**	0.002 (0.058)	9.69E-01
TLCA	0.298 (-0.180)	5.09E-02	-0.008 (0.064)	9.05E-01
TLCA-S	0.049 (-0.110)	1.81E-01	-0.057 (0.060)	3.39E-01
TUDCA	0.145 (0.051)	5.92E-01	-0.082 (0.055)	1.35E-01
UDCA	0.956 (0.026)	8.30E-01	-0.018 (0.067)	7.91E-01
Lipids				
HDL cholesterol	0.315 (-0.020)	8.15E-01	-0.049 (0.054)	3.68E-01
LDL cholesterol	0.011 (-0.153)	6.94E-02	-0.098 (0.054)	6.98E-02
TG	0.058 (-0.080)	3.44E-01	-0.082 (0.056)	1.42E-01
Phospholipids				
LysoPC a C14:0	0.222 (-0.050)	5.98E-01	0.080 (0.052)	1.26E-01
LysoPC a C16:0	0.502 (-0.138)	1.11E-01	0.079 (0.054)	1.44E-01
LysoPC a C16:1	0.020 (-0.041)	6.67E-01	0.138 (0.052)	**8.70E-03**
LysoPC a C17:0	0.830 (-0.107)	1.78E-01	0.043 (0.057)	4.50E-01
LysoPC a C18:0	0.988 (-0.164)	**4.79E-02**	0.054 (0.055)	3.29E-01
LysoPC a C18:1	0.232 (-0.108)	2.67E-01	0.079 (0.053)	1.35E-01
LysoPC a C18:2	0.558 (-0.105)	2.49E-01	0.056 (0.054)	2.95E-01
LysoPC a C18:3	0.560 (-0.054)	5.28E-01	0.051 (0.054)	3.49E-01
LysoPC a C20:3	0.781 (-0.188)	**2.60E-02**	0.071 (0.054)	1.89E-01
LysoPC a C20:4	0.873 (-0.129)	1.82E-01	0.024 (0.054)	6.59E-01
LysoPC a C20:5	0.706 (-0.096)	2.11E-01	0.008 (0.057)	8.84E-01
LysoPC a C22:5	0.775 (-0.050)	5.33E-01	0.035 (0.056)	5.38E-01
LysoPC a C22:6	0.735 (-0.114)	1.83E-01	0.008 (0.054)	8.77E-01
LysoPC a C24:0	0.210 (0.086)	3.49E-01	0.053 (0.052)	3.15E-01
LysoPC a C26:0	0.557 (-0.020)	8.41E-01	0.026 (0.052)	6.14E-01
LysoPC a C26:1	0.465 (0.081)	3.75E-01	0.003 (0.053)	9.56E-01
LysoPC a C28:0	0.898 (-0.124)	1.41E-01	0.032 (0.055)	5.63E-01

LysoPC a C28:1	0.424 (-0.125)	1.18E-01	-0.004 (0.056)	9.40E-01
PC aa C24:0	0.480 (0.057)	4.55E-01	0.008 (0.058)	8.96E-01
PC aa C26:0	0.553 (-0.048)	5.85E-01	0.037 (0.056)	5.15E-01
PC aa C28:1	0.547 (-0.013)	8.73E-01	-0.025 (0.056)	6.49E-01
PC aa C30:0	0.923 (0.029)	7.41E-01	-0.005 (0.053)	9.29E-01
PC aa C32:0	0.912 (0.013)	8.81E-01	-0.008 (0.053)	8.77E-01
PC aa C32:1	0.476 (0.075)	4.13E-01	0.027 (0.053)	6.04E-01
PC aa C32:3	0.586 (0.019)	8.26E-01	-0.034 (0.054)	5.22E-01
PC aa C34:1	0.726 (-0.005)	9.57E-01	-0.016 (0.054)	7.60E-01
PC aa C34:2	0.505 (-0.036)	6.89E-01	-0.023 (0.053)	6.60E-01
PC aa C34:3	0.544 (-0.021)	8.33E-01	-0.023 (0.052)	6.62E-01
PC aa C34:4	0.742 (0.000)	9.98E-01	-0.016 (0.052)	7.56E-01
PC aa C36:0	0.162 (-0.042)	6.37E-01	-0.068 (0.057)	2.29E-01
PC aa C36:1	0.857 (-0.021)	8.14E-01	0.003 (0.054)	9.56E-01
PC aa C36:2	0.515 (-0.051)	5.75E-01	-0.017 (0.053)	7.49E-01
PC aa C36:3	0.895 (-0.072)	3.78E-01	0.027 (0.055)	6.26E-01
PC aa C36:4	0.497 (-0.023)	8.01E-01	-0.036 (0.053)	4.95E-01
PC aa C36:5	0.403 (-0.014)	8.52E-01	-0.040 (0.057)	4.85E-01
PC aa C36:6	0.538 (-0.029)	7.57E-01	-0.023 (0.053)	6.74E-01
PC aa C38:0	0.266 (-0.056)	5.11E-01	-0.039 (0.056)	4.83E-01
PC aa C38:1	0.735 (0.015)	9.01E-01	-0.028 (0.070)	6.94E-01
PC aa C38:3	0.592 (-0.110)	1.88E-01	0.015 (0.055)	7.83E-01
PC aa C38:4	0.275 (-0.068)	4.69E-01	-0.048 (0.053)	3.63E-01
PC aa C38:5	0.962 (0.050)	5.75E-01	-0.004 (0.054)	9.37E-01
PC aa C38:6	0.201 (-0.041)	6.44E-01	-0.058 (0.055)	2.93E-01
PC aa C40:1	0.656 (-0.123)	1.85E-01	0.023 (0.054)	6.73E-01
PC aa C40:2	0.361 (0.137)	1.03E-01	0.007 (0.055)	8.95E-01
PC aa C40:3	0.971 (-0.012)	8.91E-01	0.009 (0.054)	8.66E-01
PC aa C40:4	0.628 (-0.044)	6.45E-01	-0.005 (0.053)	9.25E-01
PC aa C40:5	0.961 (0.015)	8.56E-01	0.010 (0.056)	8.64E-01
PC aa C40:6	0.153 (-0.063)	4.94E-01	-0.058 (0.055)	2.89E-01
PC aa C42:0	0.189 (-0.133)	1.52E-01	-0.024 (0.055)	6.58E-01
PC aa C42:1	0.260 (-0.064)	4.63E-01	-0.039 (0.054)	4.76E-01
PC aa C42:2	0.150 (-0.080)	4.09E-01	-0.061 (0.052)	2.39E-01
PC aa C42:4	0.394 (-0.093)	3.16E-01	-0.021 (0.053)	6.93E-01
PC aa C42:5	0.188 (-0.113)	2.63E-01	-0.044 (0.051)	3.89E-01
PC aa C42:6	0.441 (-0.050)	6.08E-01	-0.027 (0.053)	6.09E-01
PC ae C30:0	0.686 (0.020)	8.15E-01	-0.020 (0.055)	7.13E-01
PC ae C30:1	0.509 (0.044)	7.09E-01	-0.183 (0.237)	4.46E-01
PC ae C30:2	0.761 (-0.122)	1.71E-01	0.066 (0.054)	2.18E-01
PC ae C32:1	0.898 (-0.009)	9.13E-01	0.002 (0.054)	9.69E-01
PC ae C32:2	0.999 (-0.006)	9.47E-01	0.010 (0.054)	8.56E-01
PC ae C34:0	0.672 (-0.003)	9.68E-01	-0.011 (0.056)	8.48E-01
PC ae C34:1	0.979 (-0.003)	9.76E-01	0.005 (0.053)	9.24E-01

PC ae C34:2	0.422 (-0.057)	5.22E-01	-0.025 (0.053)	6.37E-01
PC ae C34:3	0.184 (-0.015)	8.74E-01	-0.064 (0.054)	2.41E-01
PC ae C36:0	0.516 (-0.003)	9.72E-01	-0.037 (0.053)	4.93E-01
PC ae C36:1	0.449 (-0.021)	8.05E-01	-0.027 (0.055)	6.21E-01
PC ae C36:2	0.476 (-0.037)	6.83E-01	-0.019 (0.054)	7.24E-01
PC ae C36:3	0.705 (-0.115)	1.89E-01	0.026 (0.054)	6.31E-01
PC ae C36:4	0.728 (-0.067)	4.57E-01	0.005 (0.053)	9.20E-01
PC ae C36:5	0.385 (-0.037)	6.58E-01	-0.037 (0.055)	5.04E-01
PC ae C38:0	0.482 (-0.004)	9.61E-01	-0.036 (0.055)	5.18E-01
PC ae C38:1	0.171 (0.003)	9.73E-01	-0.071 (0.054)	1.96E-01
PC ae C38:2	0.644 (0.046)	5.84E-01	-0.027 (0.058)	6.38E-01
PC ae C38:3	0.676 (-0.062)	4.68E-01	0.014 (0.056)	8.06E-01
PC ae C38:4	0.390 (-0.084)	3.53E-01	-0.018 (0.053)	7.40E-01
PC ae C38:5	0.756 (-0.060)	4.93E-01	0.003 (0.053)	9.49E-01
PC ae C38:6	0.236 (-0.070)	4.07E-01	-0.039 (0.055)	4.74E-01
PC ae C40:1	0.604 (0.029)	7.53E-01	0.013 (0.053)	8.06E-01
PC ae C40:2	0.980 (0.037)	6.75E-01	-0.003 (0.055)	9.60E-01
PC ae C40:3	0.820 (0.027)	7.48E-01	0.015 (0.056)	7.84E-01
PC ae C40:4	0.821 (-0.037)	6.82E-01	0.011 (0.054)	8.34E-01
PC ae C40:5	0.666 (-0.037)	6.86E-01	-0.007 (0.053)	9.02E-01
PC ae C40:6	0.187 (-0.078)	3.93E-01	-0.043 (0.055)	4.41E-01
PC ae C42:0	0.934 (0.041)	6.36E-01	-0.023 (0.053)	6.70E-01
PC ae C42:1	0.755 (0.017)	8.56E-01	0.012 (0.052)	8.17E-01
PC ae C42:2	0.622 (-0.018)	8.42E-01	0.034 (0.052)	5.14E-01
PC ae C42:3	0.903 (0.011)	8.95E-01	0.001 (0.054)	9.89E-01
PC ae C42:4	0.393 (-0.084)	3.49E-01	-0.019 (0.054)	7.30E-01
PC ae C42:5	0.542 (-0.078)	3.81E-01	-0.003 (0.054)	9.54E-01
PC ae C44:3	0.929 (0.009)	9.31E-01	-0.017 (0.052)	7.50E-01
PC ae C44:4	0.208 (-0.073)	4.40E-01	-0.052 (0.053)	3.24E-01
PC ae C44:5	0.438 (-0.111)	2.22E-01	-0.009 (0.053)	8.69E-01
PC ae C44:6	0.373 (-0.063)	4.92E-01	-0.029 (0.053)	5.84E-01
SM (OH) C14:1	0.281 (-0.096)	2.68E-01	-0.016 (0.056)	7.78E-01
SM (OH) C16:1	0.237 (-0.103)	2.38E-01	-0.020 (0.056)	7.15E-01
SM (OH) C22:1	0.752 (-0.026)	7.45E-01	0.062 (0.059)	2.93E-01
SM (OH) C22:2	0.925 (-0.046)	5.76E-01	0.035 (0.057)	5.39E-01
SM (OH) C24:1	0.783 (-0.039)	6.31E-01	0.022 (0.059)	7.06E-01
SM C16:0	0.394 (-0.097)	2.67E-01	-0.006 (0.054)	9.10E-01
SM C16:1	0.826 (-0.059)	5.08E-01	0.015 (0.053)	7.76E-01
SM C18:0	0.451 (-0.135)	1.21E-01	0.003 (0.053)	9.59E-01
SM C18:1	0.606 (-0.097)	2.77E-01	0.000 (0.053)	9.94E-01
SM C20:2	0.487 (-0.081)	3.51E-01	-0.008 (0.055)	8.90E-01
SM C22:3	0.895 (-0.118)	1.45E-01	0.033 (0.057)	5.62E-01
SM C24:0	0.578 (-0.085)	3.30E-01	0.016 (0.056)	7.69E-01
SM C24:1	0.607 (-0.078)	3.65E-01	0.006 (0.054)	9.04E-01

SM C26:0	0.607 (-0.100)	2.70E-01	0.022 (0.056)	6.93E-01
SM C26:1	0.779 (-0.118)	1.95E-01	0.043 (0.055)	4.39E-01
Ratios	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
(AC C16:0 + AC C18:0) / AC C0	0.610 (-0.022)	7.96E-01	0.046 (0.054)	4.00E-01
(AC C2:0 + AC C3:0) / AC C0	0.244 (0.029)	7.22E-01	-0.096 (0.055)	8.43E-02
AC C12:0 / AC C10:0	0.662 (0.074)	3.37E-01	0.008 (0.059)	8.94E-01
AC C14:0 / AC C16:1	0.903 (0.045)	5.62E-01	-0.004 (0.058)	9.42E-01
AC C2:0 / AC C0	0.218 (0.025)	7.60E-01	-0.095 (0.055)	8.30E-02
AC C3:0 / AC C4:0	0.526 (-0.099)	2.32E-01	-0.005 (0.056)	9.34E-01
Amino acids				
Cit / Arg	0.024 (0.109)	2.32E-01	0.101 (0.054)	6.33E-02
Cit / Orn	0.636 (-0.039)	6.37E-01	0.038 (0.056)	5.00E-01
Fisher	0.777 (0.053)	5.53E-01	-0.009 (0.054)	8.72E-01
Gly / PC ae C38:2	0.662 (-0.022)	7.98E-01	0.027 (0.056)	6.26E-01
Orn / Arg	0.041 (0.115)	1.87E-01	0.086 (0.054)	1.12E-01
Orn / Ser	0.160 (0.088)	3.06E-01	0.055 (0.054)	3.12E-01
Tyr / Phe	0.800 (0.018)	8.39E-01	-0.035 (0.054)	5.12E-01
Bile acids				
Conj. BA / unconj. BA	0.924 (0.108)	2.04E-01	-0.026 (0.055)	6.38E-01
Prim. BA / sec. BA	0.608 (0.133)	8.95E-02	-0.022 (0.057)	7.02E-01
Phospholipids				
LysoPC / PC	0.126 (-0.096)	3.10E-01	0.102 (0.052)	5.37E-02
MUFA PC / SFA PC	0.666 (0.002)	9.80E-01	0.028 (0.062)	6.44E-01
PC aa C36:3 / PC aa C36:4	0.589 (-0.072)	4.06E-01	0.073 (0.056)	1.94E-01
PC aa C40:3 / PC aa C42:5	0.126 (0.098)	2.98E-01	0.060 (0.052)	2.49E-01
PC ae C32:1 / PC ae C34:1	0.811 (-0.012)	8.68E-01	-0.007 (0.060)	9.01E-01
PC ae C38:1 / PC aa C28:1	0.223 (0.012)	8.90E-01	-0.076 (0.056)	1.78E-01
PC ae C44:5 / PC ae C42:5	0.427 (-0.132)	1.59E-01	-0.018 (0.053)	7.31E-01
PUFA-PC / MUFA-PC	0.356 (-0.067)	4.42E-01	-0.034 (0.056)	5.43E-01
PUFA-PC / SFA-PC	0.541 (-0.064)	4.30E-01	-0.015 (0.055)	7.90E-01
SM (OH) C24:1 / SM C16:0	0.603 (0.029)	7.05E-01	0.036 (0.058)	5.33E-01
Sums	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Acyl-carnitines				
AC C16:0 + AC C18:0	0.553 (0.023)	7.94E-01	0.033 (0.053)	5.40E-01
AC C2:0 + AC C3:0	0.349 (0.038)	6.33E-01	-0.086 (0.055)	1.21E-01
Carnitines	0.380 (0.018)	8.35E-01	-0.065 (0.054)	2.30E-01
Even. carn	0.461 (0.032)	7.13E-01	-0.060 (0.054)	2.70E-01
Long. carn	0.624 (0.036)	6.89E-01	0.023 (0.053)	6.68E-01
Medium. carn	0.957 (0.030)	7.56E-01	0.005 (0.053)	9.21E-01
Odd. carn	0.490 (0.048)	5.82E-01	-0.061 (0.054)	2.57E-01
Amino acids				
AA	0.493 (0.093)	2.23E-01	0.021 (0.059)	7.22E-01
Arom. AA	0.655 (-0.046)	6.21E-01	-0.010 (0.053)	8.45E-01

BCAA	0.834 (0.014)	8.70E-01	-0.026 (0.055)	6.42E-01
Ess. AA	0.219 (0.084)	2.63E-01	0.044 (0.058)	4.49E-01
Glucog. AA	0.740 (0.052)	5.45E-01	-0.021 (0.058)	7.22E-01
Noness. AA	0.946 (0.076)	3.48E-01	-0.004 (0.059)	9.44E-01
Bile acids				
Conj. BA	0.107 (-0.084)	2.77E-01	-0.063 (0.060)	2.93E-01
Prim. BA	0.109 (-0.085)	2.70E-01	-0.062 (0.060)	3.05E-01
Sec. BA	0.180 (-0.224)	**5.78E-03**	0.002 (0.055)	9.73E-01
Unconj. BA	0.512 (-0.183)	**2.11E-02**	0.029 (0.056)	6.09E-01
Phospholipids				
Long. PC	0.259 (-0.055)	5.51E-01	-0.041 (0.054)	4.56E-01
Long. PC aa	0.221 (-0.053)	5.68E-01	-0.047 (0.054)	3.88E-01
Long. PC ae	0.585 (-0.044)	6.30E-01	-0.011 (0.054)	8.39E-01
Long. SM	0.592 (-0.085)	3.29E-01	0.011 (0.054)	8.36E-01
Long. SM C	0.590 (-0.085)	3.27E-01	0.011 (0.054)	8.41E-01
Long. SM OH	0.783 (-0.039)	6.31E-01	0.022 (0.059)	7.06E-01
LysoPC	0.489 (-0.159)	7.20E-02	0.081 (0.053)	1.32E-01
Mono. PC	0.813 (-0.002)	9.78E-01	-0.009 (0.054)	8.66E-01
Mono. PC aa	0.815 (-0.002)	9.85E-01	-0.009 (0.054)	8.62E-01
Mono. PC ae	0.842 (-0.005)	9.56E-01	-0.003 (0.053)	9.50E-01
PC	0.449 (-0.037)	6.70E-01	-0.028 (0.054)	6.02E-01
PC aa	0.445 (-0.035)	6.84E-01	-0.029 (0.054)	5.85E-01
PC ae	0.533 (-0.048)	5.85E-01	-0.014 (0.054)	8.02E-01
Poly. PC	0.407 (-0.043)	6.17E-01	-0.030 (0.054)	5.75E-01
Poly. PC aa	0.403 (-0.041)	6.31E-01	-0.032 (0.054)	5.56E-01
Poly. PC ae	0.501 (-0.056)	5.26E-01	-0.014 (0.054)	7.97E-01
Sat. LysoPC	0.569 (-0.143)	9.44E-02	0.076 (0.054)	1.60E-01
Sat. PC	0.659 (-0.003)	9.67E-01	-0.020 (0.054)	7.09E-01
Sat. PC aa	0.703 (-0.004)	9.63E-01	-0.017 (0.054)	7.52E-01
Sat. PC ae	0.514 (0.000)	9.97E-01	-0.031 (0.055)	5.67E-01
Saturmono. PC	0.813 (-0.002)	9.78E-01	-0.009 (0.054)	8.66E-01
Short. PC	0.629 (-0.028)	7.60E-01	-0.016 (0.053)	7.60E-01
Short. PC aa	0.631 (-0.027)	7.66E-01	-0.016 (0.053)	7.56E-01
Short. PC ae	0.722 (-0.004)	9.64E-01	-0.012 (0.054)	8.30E-01
SM	0.513 (-0.096)	2.67E-01	0.005 (0.054)	9.29E-01
SM (OH)	0.772 (-0.054)	5.09E-01	0.028 (0.057)	6.29E-01
SM C	0.497 (-0.099)	2.55E-01	0.003 (0.054)	9.57E-01
Unsat. lysoPC	0.398 (-0.142)	1.35E-01	0.071 (0.053)	1.78E-01
Very. lysoPC	0.560 (-0.073)	4.13E-01	0.044 (0.054)	4.13E-01

Data are β-estimate (SE) of BMI z-scores at ages one, two, three, and four years per metabolite, sums and ratios in offspring of normal weight and offspring of obese (preconception BMI ≥ 29.5 kg/m^2) mothers. Concentrations of HDL cholesterol, LDL cholesterol and TG are provided in mg/dL. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear mixed effect models adjusted for GWG, smoking during pregnancy, breastfeeding, and SES (Model 1) performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded

from the mixed effect models. Bold font indicates p-value < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. BMI, body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status; TG, triglyceride; UCB, umbilical cord blood.

Table S 13: Sensitivity analysis of significant associations (p-value <0.05) between UCB metabolites and BMI z-scores at age one, two, three, and four years using multiple mixed effect models

	Model 1a		Model 2				Model 3			
	All		Offspring of normal weight mothers		Offspring of obese mothers		Offspring of normal weight mothers		Offspring of obese mothers	
Metabolites	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Pro	0.091 (0.046)	**4.94E-02**	0.174 (0.080)	**3.31E-02**	0.061 (0.056)	2.77E-01	0.163 (0.081)	**4.72E-02**	0.071 (0.056)	2.04E-01
BAIB	0.101 (0.046)	**2.86E-02**	0.072 (0.075)	3.36E-01	0.114 (0.058)	5.12E-02	0.061 (0.076)	4.28E-01	0.092 (0.058)	1.11E-01
AAB	-0.082 (0.045)	7.08E-02	0.007 (0.091)	9.35E-01	-0.115 (0.053)	**3.07E-02**	-0.015 (0.088)	8.64E-01	-0.126 (0.054)	**1.99E-02**
AC 2-M-C4:0	-0.058 (0.047)	2.13E-01	-0.174 (0.085)	**4.26E-02**	-0.018 (0.056)	7.52E-01	-0.196 (0.081)	**1.76E-02**	-0.051 (0.056)	3.66E-01
GCA	-0.148 (0.047)	**1.61E-03**	-0.125 (0.075)	1.02E-01	-0.158 (0.061)	**9.76E-03**	-0.101 (0.074)	1.79E-01	-0.111 (0.061)	7.00E-02
TCA	-0.137 (0.046)	**3.13E-03**	-0.081 (0.075)	2.86E-01	-0.167 (0.060)	**5.65E-03**	-0.063 (0.075)	3.99E-01	-0.114 (0.060)	5.73E-02
DCA	-0.039 (0.048)	4.12E-01	-0.216 (0.083)	**1.04E-02**	0.033 (0.058)	5.67E-01	-0.220 (0.080)	**7.48E-03**	0.040 (0.059)	4.99E-01
GDCA	-0.085 (0.046)	6.53E-02	-0.272 (0.078)	**7.81E-04**	-0.001 (0.056)	9.90E-01	-0.278 (0.077)	**4.93E-04**	0.025 (0.056)	6.57E-01
TDCA	-0.052 (0.047)	2.67E-01	-0.187 (0.082)	**2.46E-02**	0.007 (0.058)	9.03E-01	-0.179 (0.083)	**3.30E-02**	0.037 (0.057)	5.24E-01
GUDCA	-0.132 (0.046)	**4.44E-03**	-0.194 (0.089)	**3.16E-02**	-0.106 (0.055)	5.29E-02	-0.194 (0.086)	**2.59E-02**	-0.099 (0.055)	7.17E-02
LysoPCaC16:1	0.101 (0.046)	**2.70E-02**	-0.040 (0.095)	6.73E-01	0.131 (0.053)	**1.33E-02**	-0.133 (0.090)	1.41E-01	0.084 (0.053)	1.17E-01
LysoPCaC18:0	-0.005 (0.046)	9.08E-01	-0.164 (0.082)	**4.87E-02**	0.054 (0.055)	3.31E-01	-0.155 (0.083)	6.37E-02	0.055 (0.055)	3.16E-01
LysoPCaC20:3	0.007 (0.046)	8.84E-01	-0.188 (0.084)	**2.64E-02**	0.068 (0.054)	2.11E-01	-0.213 (0.076)	**5.88E-03**	0.016 (0.056)	7.80E-01
Ratios and sums										
Cit / Arg	0.095 (0.046)	**3.88E-02**	0.109 (0.091)	2.33E-01	0.095 (0.054)	8.12E-02	0.098 (0.088)	2.65E-01	0.090 (0.054)	9.64E-02
Sec. BA	-0.061 (0.045)	1.80E-01	-0.224 (0.080)	**5.89E-03**	0.003 (0.055)	9.59E-01	-0.224 (0.077)	**4.61E-03**	0.020 (0.055)	7.15E-01
Unconj. BA	-0.035 (0.046)	4.47E-01	-0.183 (0.078)	**2.13E-02**	0.028 (0.056)	6.13E-01	-0.192 (0.077)	**1.44E-02**	0.036 (0.056)	5.29E-01

Data are β-estimate (SE) of BMI z-scores at ages one, two, three, and four years per metabolite, sums and ratios of significant findings in offspring of normal weight and those of obese (preconception BMI ≥ 29.5 kg/m^2) mothers analyzed in different linear mixed effect models, modified from Model 1, performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the mixed effect models. Bile acids are given in ng/mL, all other metabolites in µmol/L. Bold font indicates p-value < 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2.

Model 1: BMI z-score slope ~ metabolite (scaled) + age (years) + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + SES; Random effect: 1|ID

Model 1a: BMI z-score slope ~ metabolite (scaled) + age (years) + maternal preconception BMI (normal weight and obese, ≥29.5 kg/m2) + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + SES; Random effect: 1|ID

Model 2: BMI z-score slope ~ metabolite (scaled) + age (years) + maternal HbA1c (%) at delivery + GWG + smoking anytime during pregnancy + breastfeeding (predominant ≥1 month) + parental SES at birth; Random effect: 1|ID

Model 3: BMI z-score slope~ metabolite (residuals) + age (years) + GWG + smoking anytime during pregnancy breastfeeding (predominant ≥1 month) + parental SES at birth; Random effect: 1|ID

Metabolite (residuals) presents the value after correcting the metabolite log2-transformed concentrations for potential confounding factors used to investigate association between UCB metabolites and maternal obesity (Table S 5); Metabolite (scaled) presents the log2-transformed metabolite concentrations scaled to a mean of 0 and SD of 1; BMI, body mass index; GWG, gestational weight gain; SE; standard error; SES, socio-economic status UCB, umbilical cord blood.

Table S 14: Significant associations (p < 0.05) of UCB metabolites and BMI z-scores at ages one, two, three, and four years according to maternal BMI group and offspring sex

	Offspring of normal weight mothers						Offspring of obese mothers					
	All		Male		Female		All		Male		Female	
Metabolite	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**	**β-estimate (SE)**	**p-Value**
Pro	0.173 (0.080)	**3.36E-02**	0.013 (0.085)	**1.65E-02**	0.073 (0.075)	2.75E-01	0.060 (0.056)	2.91E-01	0.013 (0.085)	8.76E-01	0.073 (0.075)	3.36E-01
BAIB	0.072 (0.075)	3.35E-01	0.203 (0.083)	8.36E-01	0.017 (0.081)	3.41E-01	0.118 (0.058)	**4.37E-02**	0.203 (0.083)	**1.63E-02**	0.017 (0.081)	8.31E-01
AAB	0.009 (0.090)	9.24E-01	-0.138 (0.074)	1.88E-01	-0.118 (0.078)	4.17E-01	-0.113 (0.053)	**3.35E-02**	-0.138 (0.074)	6.24E-02	-0.118 (0.078)	1.33E-01
AC 2-M-C4:0	-0.173 (0.084)	**4.19E-02**	0.009 (0.086)	7.92E-01	-0.066 (0.074)	**2.86E-02**	-0.013 (0.056)	8.15E-01	0.009 (0.086)	9.16E-01	-0.066 (0.074)	3.77E-01
GCA	-0.124 (0.075)	1.01E-01	-0.203 (0.099)	**4.64E-02**	-0.049 (0.107)	6.52E-01	-0.162 (0.061)	**8.42E-03**	-0.207 (0.095)	**3.04E-02**	-0.106 (0.078)	1.76E-01
TCA	-0.081 (0.075)	2.85E-01	-0.184 (0.088)	1.52E-01	-0.136 (0.081)	9.31E-01	-0.166 (0.060)	**6.12E-03**	-0.184 (0.088)	**3.87E-02**	-0.136 (0.081)	9.57E-02
DCA	-0.216 (0.082)	**1.03E-02**	0.026 (0.090)	2.50E-01	0.058 (0.076)	**1.97E-02**	0.025 (0.058)	6.61E-01	0.026 (0.090)	7.69E-01	0.058 (0.076)	4.49E-01
GDCA	-0.271 (0.078)	**7.75E-04**	-0.028 (0.088)	**4.16E-02**	0.021 (0.072)	**6.48E-03**	-0.011 (0.056)	8.50E-01	-0.028 (0.088)	7.53E-01	0.021 (0.072)	7.66E-01
TDCA	-0.187 (0.082)	**2.44E-02**	0.010 (0.090)	8.32E-02	0.015 (0.076)	5.90E-02	0.002 (0.058)	9.69E-01	0.010 (0.090)	9.13E-01	0.015 (0.076)	8.43E-01
GUDCA	-0.193 (0.089)	**3.14E-02**	-0.181 (0.079)	**3.57E-03**	-0.021 (0.078)	9.16E-01	-0.107 (0.055)	5.05E-02	-0.181 (0.079)	**2.29E-02**	-0.021 (0.078)	7.89E-01
LysoPC a C16:1	-0.041 (0.095)	6.67E-01	0.193 (0.075)	8.45E-01	0.066 (0.073)	2.50E-01	0.138 (0.052)	**8.70E-03**	0.193 (0.075)	**1.18E-02**	0.066 (0.073)	3.68E-01
LysoPC a C18:0	-0.164 (0.082)	**4.79E-02**	0.047 (0.085)	4.48E-01	0.045 (0.074)	**7.96E-03**	0.054 (0.055)	3.29E-01	0.047 (0.085)	5.82E-01	0.045 (0.074)	5.46E-01
LysoPC a C20:3	-0.188 (0.083)	**2.60E-02**	0.120 (0.078)	2.10E-01	0.015 (0.076)	5.62E-02	0.071 (0.054)	1.89E-01	0.120 (0.078)	1.28E-01	0.015 (0.076)	8.45E-01
Ratios and Sums												
Cit / Arg	0.109 (0.091)	2.32E-01	0.175 (0.082)	1.92E-01	0.002 (0.072)	1.18E-01	0.101 (0.054)	6.33E-02	0.175 (0.082)	**3.54E-02**	0.002 (0.072)	9.78E-01
Sec. BA	-0.224 (0.080)	**5.78E-03**	-0.123 (0.089)	9.45E-02	0.118 (0.070)	**3.77E-02**	0.002 (0.055)	9.73E-01	-0.123 (0.089)	1.66E-01	0.118 (0.070)	9.32E-02
Unconj. BA	-0.183 (0.078)	**2.11E-02**	-0.068 (0.085)	**4.39E-02**	0.147 (0.074)	2.80E-01	0.029 (0.056)	6.09E-01	-0.068 (0.085)	4.23E-01	0.147 (0.074)	**4.91E-02**

Data are β-estimate (SE) of BMI z-scores at ages one, two, three, and four years per metabolite, sums and ratios in offspring of normal weight and offspring of obese (preconception BMI ≥ 29.5 kg/m^2) mothers and those stratified by offspring sex. Bile acids are given in ng/mL, all other metabolites in µmol/L. Data are based on linear mixed effect models adjusted for GWG, smoking during pregnancy, breastfeeding, and SES (Model 1) performed in n = 111 offspring of normal weight mothers and n = 287 offspring of obese mothers after the dataset was stratified by maternal BMI. Offspring with missing data on any of the potential confounder were excluded from the mixed effect models. The analysis was repeated after dataset was stratified by offspring sex. Bold font indicates p-value< 0.05. A full list of metabolite abbreviations is provided in Table S 1, of sums and ratios in Table S 2. BMI, body mass index; GWG, gestational weight gain; SE, standard error; SES, socio-economic status; UCB, umbilical cord blood

Table S 15: GUDCA sensitivity analysis

	β-estimate (SE)	p-Value
Model 1	-0.138 (0.046)	**3.20E-03**
Model II	-0.141 (0.047)	**2.70E-03**
Model III	-0.138 (0.046)	**3.20E-03**
Model IV	-0.137 (0.046)	**3.30E-03**
Model Va	-0.199 (0.054)	**3.00E-04**
Model Vb	-0.059 (0.084)	4.87E-01

Data are β-estimate (SE) of BMI z-score at one, two, three, and four years for GUDCA concentration (ng/mL) of offspring of all mothers analyzed in different linear mixed effect models performed in n = 398 offspring. Offspring with missing data on any of the potential confounder were excluded from the mixed effect models. Bold font indicates p-value < 0.05.

Model 1: BMI z-score ~ metabolite (scaled) + age (years) + GWG + smoking anytime during pregnancy + breastfeeding (predominantly ≥1 month) + parental SES at birth; Random effect: 1|ID

Model II: Model 1 + intrapartum antibiotic treatment

Model III: Model II + maternal preconception BMI (normal weight and obese, ≥29.5 kg/m^2)

Model IV: Model III + maternal HbA1c (%) at delivery

Model Va: Model 1 in breastfed offspring

Model Vb: Model 1 in non-breastfed offspring

GUDCA, glycoursodeoxycholic acid; SES, socio-economic status.

Figure S 1: Sample distribution on 96 well plates

Platte 1	1	2	3	4	5	6	7	8	9	10	11	12
A	1043	1274	2077	2157	2345	4017	4122	6060				
B	1048	1280	2114	2169	3097	4054	4133	6068				
C	1131	1284	2116	2180	3109	4062	4140	6075				
D	1156	1352	2121	2189	3118	4064	4144	6076				
E	1231	1369	2126	2192	3139	4071	6036					
F	1232	1393	2142	2199	3149	4072	6047					
G	1241	1407	2152	2205	3169	4092	6056					
H	1247	2011	2153	2301	4013	4094	6056					

Platte 2	1	2	3	4	5	6	7	8	9	10	11	12
A	1060	1266	2165	2308	3058	4022	4143	6053				
B	1089	1285	2186	2309	3061	4069	4154	6073				
C	1095	1310	2206	2314	3129	4093	4155	6092				
D	1108	1319	2220	2315	3138	4106	5009	6100				
E	1167	1363	2250	2318	3141	4121	5282					
F	1169	1368	2254	2322	3142	4126	6018					
G	1223	2053	2297	2329	4004	4128	6024					
H	1225	2061	2307	2327	4022	4143	6038					

Platte 3	1	2	3	4	5	6	7	8	9	10	11	12
A	1062	1196	1304	1391	2123	2257	3046	4109	4131	5071	6106	
B	1066	1201	1309	2014	2145	2269	3105	4117	4131	6041		
C	1113	1202	1326	2023	2151	2278	3152	4118	4136	6046		
D	1148	1240	1327	2044	2193	2283	3153	4118	4136	6059		
E	1149	1256	1364	2055	2202	2289	3180	4119	4149	6066		
F	1157	1268	1379	2098	2216	2311	3187	4119	4168	6066		
G	1175	1287	1389	2102	2238	2320	4074	4123	5049	6080		
H	1178	1291	1390	2122	2244	3004	4109	4123	5049	6080		

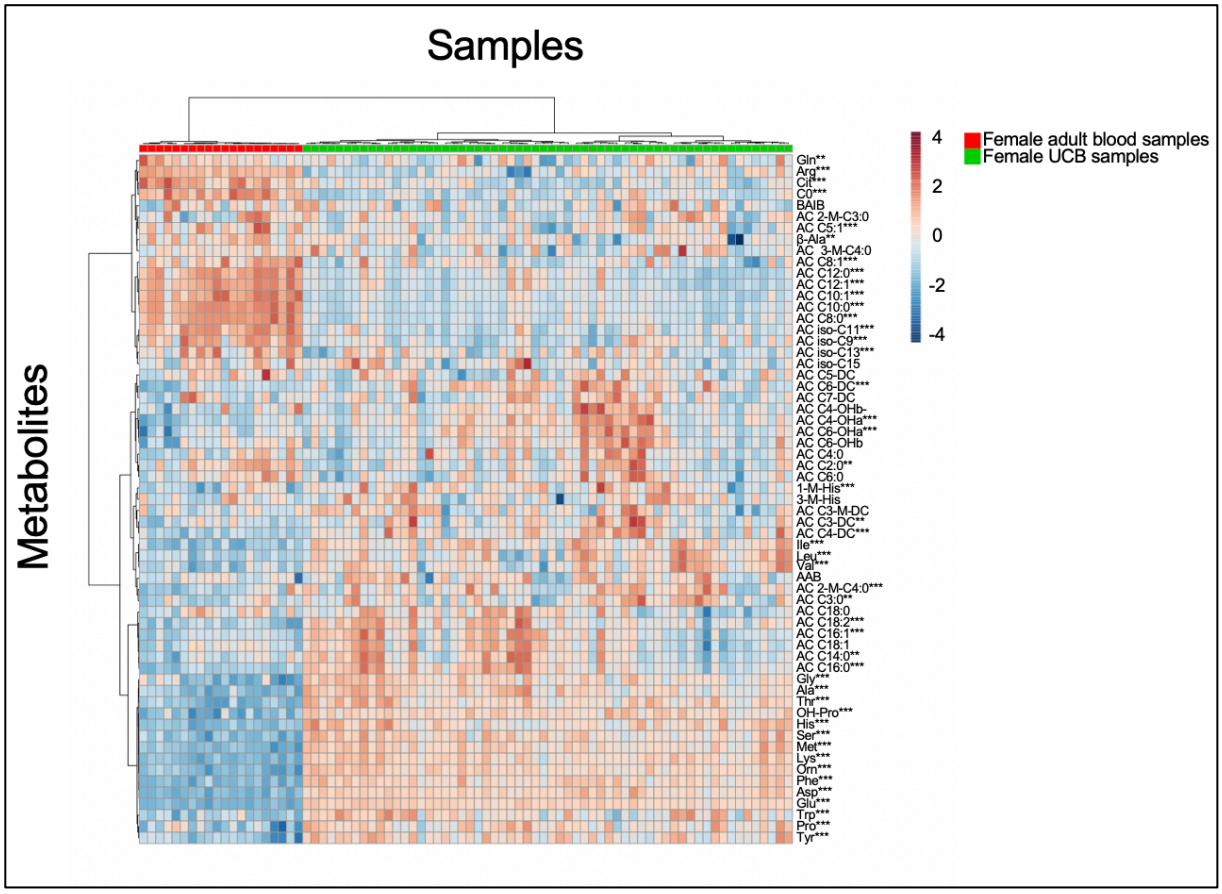
Platte 4	1	2	3	4	5	6	7	8	9	10	11	12
A	1049	1174	1298	2001	2120	2197	3096	4050	4090	5204	6094	
B	1050	1176	1299	2028	2131	2203	3103	4055	4091	6006		
C	1077	1180	1311	2030	2141	2215	3104	4055	4091	6037		
D	1107	1188	1348	2074	2143	2219	3123	4080	4107	6078		
E	1117	1237	1371	2081	2160	2221	3125	4082	4147	6078		
F	1143	1245	1376	2094	2161	2229	3171	4082	4152	6091		
G	1153	1273	1378	2108	2177	2274	4025	4089	5127	6091		
H	1164	1290	1416	2113	2194	2323	4050	4089	5136	6094		

Platte 5	1	2	3	4	5	6	7	8	9	10	11	12

A	1021	1109	1197	1359	2075	2173	3100	4051	4113			
B	1023	1111	1210	1388	2076	2222	3114	4063	4113			
C	1026	1124	1210	1394	2091	2233	4026	4063	4114			
D	1030	1147	1257	2031	2107	2239	4027	4073	4114			
E	1047	1152	1261	2040	2115	2275	4034	4078	4162			
F	1064	1154	1269	2046	2128	2287	4036	4087	6065			
G	1086	1165	1278	2052	2155	2310	4036	4087	6067			
H	1096	1173	1344	2064	2159	3086	4051	4112	6082			

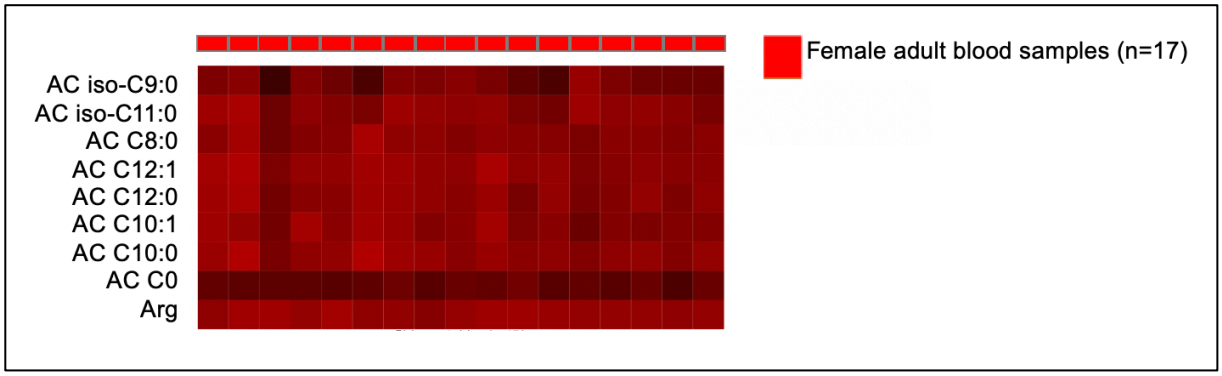
Platte 6	1	2	3	4	5	6	7	8	9	10	11	12
A	1018	1127	1250	2007	2079	2236	3107	4039	4110	6003		
B	1053	1146	1272	2013	2092	2241	3155	4056	4130	6009		
C	1068	1155	1296	2021	2095	2279	3161	4056	4135	6017		
D	1076	1166	1315	2024	2097	2280	4009	4083	4170	6020		
E	1081	1182	1355	2042	2103	3065	4029	4083	4174	6052		
F	1087	1205	1358	2047	2119	3084	4030	4085	5015	6052		
G	1110	1218	1365	2048	2129	3095	4031	4085	5169			
H	1123	1224	1401	2060	2191	3098	4033	4110	5311			

Figure S 2: **Heatmap of amino acids and acyl-carnitines in female adult blood (n = 20) and female UCB (n = 60) after hierarchical clustering**



The analysis was performed using Spearman's rank correlation as similarity measure and the average linkage as agglomeration method. Concentrations are given in µmol/L. Red represents high, blue represents low concentrations. Statistical significance is based on p-values adjusted for multiple testing by the method of Benjamini-Hochberg. * = q-value < 0.05, ** = q-value < 0.01 *** = q-value < 0.001.. * = q-value < 0.05, ** = q-value < 0.01 *** = q-value < 0.001. A full list of metabolite abbreviations is provided in Table S 1. UCB, umbilical cord blood.

Figure S 3: Bi-clustering of amino acids and acyl-carnitines based on female adult blood (n = 20) and UCB of female offspring (n = 60).



Concentrations are given in µmol/L. A full list of metabolite abbreviations is provided in Table S 1. UCB, umbilical cord blood.

Acknowledgement

My first thank goes to Prof. Dr. Regina Ensenauer and Prof. Dr. Hannelore Daniel for introducing me to the fascinating world of metabolites and fetal programming, and for giving me the opportunity to perform my PhD project, the support and guidance throughout.

This work would not have been possible without the support, assistance and encouragement of the colleagues of the PEACHES study group in Munich and Düsseldorf, who have accompanied me over the years and the new colleagues from the Max Rubner-Institut. Special thanks to Susanne Wullinger for technical assistance, to Dr. Soner Öner-Sieben for technical and scientific assistance, Dr. Christina Kunz for statistical support, and (former) colleagues and friends Delphina Gomes, Elodie Eiffener, Miriam Bißbort, Elisa Müller, Vroni Vollmuth, Lukas Ostermair, Dr. Franziska Büsing, and Sandra Reitmeier for valuable input, discussions, support and advice scientific and beyond.

Special thank goes to Dr. Gabi Kastenmüller (Institute of Computational Biology, Helmholtz Zentrum München), who introduced me to statistical approaches in the amazing field of metabolomics, for statistical assistance, valuable discussions and most of all the time she invested in my project. Also, thanks to Andreas Dunkel (Leibniz-Institute for Food Systems Biology, Technical University of Munich, Freising, Germany) for offering additional insights of statistical analysis of metabolomic datasets.

My thanks and appreciation to the members of the Nutritional Physiology Unit (TUM, Freising, Germany) for enabling the implementation of the experimental part and sharing their expertise. Special thank goes to Dr. Pieter Giesbertz, Dr. Kurt Gedrich, Barbara Gelhaus and Ronny Scheundel.

Also, I want to thank Prof. Dr. Gernot Desoye (Department of Obstetrics and Gynaecology, Medical University of Graz, Graz, Austria) for taking the time to come to Germany to discuss and support my project and for providing valuable insights into placental metabolic aspects.

Special thank goes to my parents, partner and friends, for love, patients and support throughout the years.

Publications and oral presentations

Peer-reviewed manuscripts

Stirm L, Kovarova M, Perschbacher S, Michlmaier R, Fritsche L, Siegel-Axel D, Schleicher E, Peter A, Pauluschke-Fröhlich J, Brucker S, Abele H, Wallwiener D, Preissl H, Wadsack C, Häring HU, Fritsche A, Ensenauer R, Desoye G, Staiger H. BMI-Independent Effects of Gestational Diabetes on Human Placenta. Journal Clin Endocrinol Metab. 2018;103(9):3299-309.

Accepted manuscripts

Uzan-Yulzari A*, Turta O*, Belogolovski A, Ziv O, Kunz C, Perschbacher S, Neuman H, Pasolli E, Oz A, Ben-Amram H, Kumar H, Ollila H, Kaljonen A, Segata N, Sharon I, Isolauri E, Salminen S, Lagström H, Louzoun Y, , Ensenauer R, Samuli Rautava S^{+}, Koren O^{+}. Neonatal antibiotic exposure impairs child growth during the first six years of life by perturbing intestinal microbial colonization while antibiotic exposure later in childhood is associated with increased body-mass index. (*shared first authorship,$^{+}$shared senior authorship)

Perschbacher S, Eckel N, Gomes D, Ensenauer R. Mütterliche Adipositas und langfristige Auswirkungen auf die Nachkommen. In: Strauss A, Strauss C, editor. Adipositas und Schwangerschaft. Berlin, Germany: Springer-Verlag GmbH; 2020.

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Perschbacher S*, Kastenmüller G*, Öner-Sieben S, Giesbertz P, Gomes D, Gedrich K, Desoye G, Dunkel A, Mansmann U, Roscher AA, Daniel H, Ensenauer R. Offspring metabolite profile at birth is related to the severity of maternal preconception obesity and preschool weight status. (*shared first authorship)

Perschbacher S, Kunz C, Giesbertz P, Daniel H, Hauner H, Ensenauer R. Differences in amino acid and acyl-carnitines concentrations in umbilical cord blood compared to adulthood.

Gomes D, Le L, Perschbacher S, Haas NA, Netz H, Hasbargen U, Delius M, Lange K, Nennstiel-Ratzel U, Roscher AA, Mansmann U PhD^{+}, Regina Ensenauer R^{+}. Risk prediction of early excessive growth in offspring exposed to obesity in pregnancy ($^{+}$shared senior authorship)

Oral presentation

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