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Immunometabolic consequences of overweight and nutrition on adipose tissue in pregnant individuals and their offspring

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- Gestational metabolism of bile acids is dysregulated in multiple sclerosis patients

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- Maternal overweight alters cord blood but not maternal plasma bile acid pool hydrophobicity

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µg	microgram
AA	arachidonic acid
ABCB4	ATP binding cassette subfamily B member 4
ACC	acetyl-CoA carboxylase
ALP	alkaline phosphatase
ALT	alanine transaminase
AMPK	5' AMP-activated protein kinase
APC	antigen presenting cells
AT	Adipose tissue
ATGL	adipose triglyceride lipase
BA	bile acid
BAT	brown adipose tissue
BHT	butylated hydroxytoluene
BMI	body mass index
BSA	bovine serum albumin
BSH	bile salt hydrolase
CA	cholic acid
CB	cord blood
CBA	conjugated bile acids
CD36	cluster of differentiation 36
CDCA	chenodeoxycholic acid
CE	cholesteryl ester
CER	ceramide
CerS	ceramide synthase
ChREBP	carbohydrate response element binding protein
CI	confidence interval
cm	centimeter
CoA	coenzyme A
CRP	C-reactive protein
CYP27A1	sterol 27-hydroxylase
CYP7A1	cholesterol-7α-hydroxylase
CYP7B1	oxysterol-7α-hydroxylase
CYP8B1	sterol 12α-hydroxylase
DAG	diacylglycerol

DCA	deoxycholic acid
DCER	dihydroceramide
DGAT2	diacylglycerol O-acyltransferase 2
DHA	docosahexaenoic acid
DIO2	type 2 iodothyronine deiodinase
dl	deciliter
DNL	*de novo* lipogenesis
DOHaD	Developmental Origins of Health and Disease
ELISA	Enzyme-linked Immunosorbent Assay
ELOVL-6	Fatty Acid Elongase 6
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
EtOH	ethanol
FA	fatty acid
FABP	fatty acid binding protein
FADS	fatty acid desaturase
FATP	fatty acid transport protein
FFA	free fatty acid
FGF	fibroblast growth factor
FGF15/19	fibroblast growth factor 15/19
Fig	figure
FXR	farnesoid X receptor
g	gram
GCA	glycocholic acid
GCDCA	glycochenodeoxycholic acid
GDCA	glycodeoxycholic acid
GDF15	Growth differentiation factor 15
GDM	gestational diabetes mellitus
GFRAL	GDNF family receptor α-like
GLUT	glucose transporter
HCA	hyocholic acid
HCER	hexosylceramide
HDL	high density lipoprotein
HFD	high-fat diet
HSL	hormone sensitive lipase
ICP	intrahepatic cholestasis of pregnancy

IFN-γ	interferon-γ
IL	interleukin
IRS	insulin receptor substrate
IS	internal standard
JAK2	janus kinase 2
kg	kilogram
KO	knockout
l	liter
LA	linoleic acid
LAM	lipid associated macrophage
LC	liquid chromatography
LCA	lithocholic acid
LCER	lactosylceramide
LCFA	long-chain fatty acid
LDL	low density lipoprotein
LDLR	LDL receptor
LepR	Leptin receptor
LPC	lysophosphatidylcholine
LPCAT	lysophosphatidylcholine acyltransferase
LPE	lysophosphatidylethanolamine
LPL	lipoprotein lipase
m	meter
MeOH	methanol
MFSD2A	major facilitator superfamily domain-containing protein 2a
mg	milligram
$MgCl_2$	magnesium chloride
MIC-1	macrophage inhibitory cytokine 1
min	minutes
ml	milliliter
MS	mass spectrometry
MTBE	methyl *tert*-butyl ether
mTORC1	mammalian target of rapamycin complex 1
MUFA	monounsaturated fatty acid
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NEFA	non esterified fatty acids

ng	nanogram
NH_4Ac	ammonium acetate
NK	natural killer
PBS	phosphate buffered saline
PC	phosphatidylcholine
PCA	principal component analysis
PE	phosphatidylethanolamine
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B
PKLR	pyruvate kinase L/R
PL	glycerophospholipid
PLA2	phospholipase A2
PLTP	phospholipid transfer protein
pmol	picomole
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
ROS	reactive oxygen species
rpm	revolutions per minute
s	seconds
SCD	stearoyl-CoA desaturase
SEM	standard error of the mean
SFA	saturated fatty acid
SHP	small heterodimer partner
SM	sphingomyelin
SREBP-1c	sterol regulatory element-binding protein 1c
STAT3	signal transducer and activator of transcription 3
T2DM	type 2 diabetes mellitus
TAG	triglyceride
TCA	taurocholic acid
TCDCA	taurochenodeoxycholic acid
TDCA	taurodeoxycholic acid
TGF-β	transforming growth factor-β
TGR5	Takeda G protein-coupled receptor 5
Th1 cell	T helper cell
THDCA	taurohyodeoxycholic acid
TLCA	taurolithocholic acid

TLR4	toll-like receptor 4
TNFα	tumor necrosis factor-α
T_{reg} cell	regulatory T cell
TREM2	Triggering receptor expressed on myeloid cells 2
Tri	trimester
TRL	triglyceride-rich lipoprotein
Tsc2	tuberous sclerosis complex 2
TUDCA	tauroursodeoxycholic acid
T-α-MCA	tauro-α-muricholic acid
T-β-MCA	tauro-β-muricholic acid
UBA	unconjugated bile acid
UDCA	ursodeoxycholic acid
UPLC	Ultra performance liquid chromatography
VLCFA	very long-chain fatty acid
VLDL	very low-density lipoprotein
WHO	World Health Organization
WT	wildtype
α-MCA	α-muricholic acid
β-MCA	β-muricholic acid
ω-MCA	ω-muricholic acid

Part A: Introduction

1. The transgenerational cycle of overweight

1.1 Developmental Origins of Health and Disease (DOHaD)

In the 1980s, scientists such as David Barker and Clive Osmond recognized associations between birth outcomes and heart disease in later life in epidemiological studies (Barker et al., 1993; Barker & Osmond, 1986). At that time, the idea of *in utero* programming by environmental factors was controversial. Instead, the concept of genetic inheritance and unhealthy lifestyle in adult life as causes for chronic diseases dominated in the scientific community at that time. During the following decades, the idea of pregnancy as priming time window was established and is nowadays widely accepted. This idea is summarized as the Developmental Origins of Health and Disease (DOHaD) hypothesis, which suggests that pregnancy is a critical developmental stage to modify the predisposition of offspring for diseases during adulthood (Barker, 1990, 2007). This concept was originally derived from epidemiological and observational studies, thus lacking molecular processes involved in the aetiology of *in utero* priming by that time.

The following chapters will summarize basic information on overweight as well as lipid metabolism during health and disease. Moreover, metabolic adaptions during pregnancy and basic concepts of the fetomaternal interaction will be evaluated with special attention to maternal overweight and/or obesity.

1.2 Overweight and obesity

Overweight is defined as a body mass index (BMI) greater than 25 kg/m², whereas obesity is characterized by a BMI greater than 30 kg/m². Both conditions mainly originate from a chronic surplus of dietary calories in combination with insufficient physical activity. Particularly in industrial countries, overweight represents a serious health issue and concerns a respective percentage of the world population (Haslam & James, 2005). Regarding this, obesity is a major risk factor for the development of disorders such as type 2 diabetes mellitus (T2DM), cardiovascular disease and non-alcoholic fatty liver disease (NAFLD) (Fabbrini et al., 2010; Field et al., 2001).

Healthy pregnancy is depending on a tight regulation of nutrient distribution, which is dysregulated in maternal overweight (see chapters 1.4 and 1.5, respectively). Notably, this altered gestational metabolic profile is associated with a variety of adverse health outcomes not only during gestation itself, but also beyond pregnancy on a long-term scale. Epidemiological studies suggest that offspring from mothers with overweight are more likely to develop macrosomia, heavy birth weight and childhood obesity (Ehrenberg et al., 2004; Salsberry & Reagan, 2005). This transgenerational inheritance of overweight is linked to an increased risk for the children to develop T2DM, cognitive deficits and respiratory diseases themselves (Godfrey et al., 2017; Harpsøe et al., 2013). From a perspective of the DOHaD hypothesis, the increasing prevalence of overweight in pregnant women is an urgent call to overcome this transgenerational cycle of overweight and obesity. Processes involved in the complex etiology and multifactorial development of these disorders will be described in the following sections.

1.3 Adverse effects of overweight on energy homeostasis and metabolic tissues

Functional adipose tissue (AT) serves as an energy reservoir that is replenished during times of caloric surplus and stores this energy mainly in the form of triglycerides (Rosen & Spiegelman, 2014). In periods of high energy demand, such as physical activity, thermogenesis or pregnancy, lipids can be mobilized by lipolysis to serve as fuels in these processes (Laurens, de Glisezinski, et al., 2020; Young & Zechner, 2013).

However, if prolonged overnutrition exceeds ATs capacity to store lipids, adipocytes become hypertrophic and dysfunctional. Consequently, this provokes a chronic inflammatory state and cytokines secretion, which attracts immune cells to infiltrate into the AT. Once immune cells infiltrated into the AT, their immunological identity shifts towards a pro-inflammatory phenotype with further secretion of inflammatory cytokines (reviewed in Kumari et al., 2017). As part of the interaction between immune cells and adipocytes, fat cells undergo a further expansion and an altered secretion of AT-specific cytokines, so called adipokines (reviewed in Cohen et al., 2017; Kumari et al., 2017).

1.4 Metabolic adaptions during physiological pregnancy

Pregnancy is a complex phase of both hormonal and metabolic adaptions. Already with the onset of pregnancy, the maternal metabolism undergoes extensive transformations to ensure a proper development of the growing fetus. With regard to this, early pregnancy is a crucial anabolic phase to build energy reservoirs to meet the energy demands of the feto-placental unit during later stages of gestation (Lain & Catalano, 2007). Circulating hormones, including insulin, estrogen, and progesterone, promote lipid deposition while suppressing lipolysis in AT (Butte, 2000). As pregnancy progresses, a physiological, peripheral insulin resistance is established to promote glucose availability for the placenta and fetus (Sonagra et al., 2014). Consequently, expression of Glucose transporter 4 (GLUT4) protein in AT is downregulated during physiological human pregnancy, thus inhibiting glucose clearance by adipocytes, and favoring maternal hyperglycemia and transplacental transport of glucose to the fetus (Okuno et al., 1995). In contrast, late pregnancy is characterized by an increased breakdown of lipid stores in AT, as indicated by increased lipolytic gene expression (Chaves & Herrera, 1980; Martin-Hidalgo et al., 1994). Hence, maternal tissues alter their metabolic profile to promote energy supply for the fetus.

1.5 Overweight and obesity during pregnancy

In parallel to the prevalence of overweight in the global population, the abundance of women at reproductive age suffering from overweight is alarming (Callaway et al., 2006; Huda et al., 2010). Thus, maternal overweight as an additional metabolic challenge has been shown to increase the risk for a variety of gestational complications, including macrosomia, preeclampsia, and preterm birth (Catalano & Ehrenberg, 2006; Ehrenberg et al., 2004). Generally, maternal obesity is characterized by hyperlipidemia and an altered fatty acid (FA) profile (Scifres et al., 2014). Moreover, placentas from women with obesity demonstrate an increased macrophage abundancy and subsequent pro-inflammatory gene signature (Challier et al., 2008). This placental inflammatory state was also reflected by higher circulating pro-inflammatory cytokines such as C-reactive protein (CRP) and interleukin-6 (IL6) (Challier et al., 2008).

1.6 Immunological adaptions during pregnancy

Immune cells play an important role in protecting the host from pathogens. During infection and subsequent inflammation, cells of the adaptive and innate immune system infiltrate into tissues of pathogenic or allogeneic antigens (Nicholson, 2016). Since the fetus inherits genetic information from both parents, it expresses paternal antigens potentially triggering maternal inflammatory responses and subsequent fetal rejection. Importantly, the existence of the placenta is not sufficient to explain the shielding of the fetus from the maternal immune repertoire as it is in immediate physical contact with the maternal decidua and, subsequently, its immunological equipment (reviewed in Arck & Hecher, 2013). Therefore, the maternal body undergoes profound changes during gestation, characterized by its remarkable immune tolerance against the fetal antigens to ensure development of the growing fetus. This adaption includes a large variety of immune cells, e.g. natural killer (NK) cells, monocytes and T cells (reviewed in Thiele et al., 2018). Mouse studies have shown that differentiation of conventional T cells into cytotoxic effector T cells in pregnancy is not directly triggered by fetal antigens, but rather through antigen presenting cells (APC) (Erlebacher et al., 2007; Kahn & Baltimore, 2010). Notably, fetal antigen-specific regulatory T (T_{reg}) cells expand over 100-fold during murine pregnancy and are derived both from peripheral conventional T cells and proliferation of already present T_{reg} cells (Rowe et al., 2012). Conclusively, physiological pregnancies are characterized by changes of the immune cell repertoire to establish tolerance of fetal antigens.

1.7 Transplacental transport of lipids

The placenta is a key organ in the fetomaternal interaction as it provides a physical barrier between mother and the growing fetus. It is also a buffering tissue for increased nutrients, as excess lipids can be internalized and selectively released into the fetal circulation if needed. However, free fatty acids are thought to passively pass the placenta (Berghaus et al., 1998). In contrast, other lipids can be provided by the mother and taken up actively by the placenta from the maternal circulation. As such, especially essential FAs are enriched in the fetus, suggesting an actively enhanced transport to the fetus (Berghaus et al., 1998; Larqué et al., 2003).

However, more complex lipids are transported by lipoprotein particles (see Figure 1, Islam et al. (2016)). Lipoproteins consist of both lipids and apolipoproteins. The core of lipoproteins mainly consists of hydrophobic lipids such as triglycerides (TAG) and cholesteryl esters (CE), whereas the hydrophilic shell consists of amphiphilic lipids such as sphingomyelins, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and free cholesterol (Feingold, 2022). Placental endothelial cells, i.e. syncytiotrophoblasts, have the capacity to hydrolyse and take up lipoprotein particles. These processes are mediated by different lipases and lipoprotein receptors, including phospholipase A2 (PLA2), lipoprotein lipase (LPL), scavenger receptor BI (SR-BI) and low-density lipoprotein receptor (LDLR) (Stadler et al., 2021). The expression of the respective proteins is mainly determined by the maternal endocrine, inflammatory, and metabolic environment. Thus, during maternal obesity there is increased placental storage of lipids (e.g. TAG), which is associated with higher cord blood lipids and altered placental genes implicated into lipid metabolism (Cetin et al., 2012; L. Song et al., 2022). Additionally, placental trophoblast cells could use the lipids either for endogenous energy homeostasis or to produce hormones for pregnancy maintenance (reviewed in Brett et al., 2014; Duttaroy, 2009). Notably, mouse studies demonstrated that placentas from females and males act differently in maternal HFD feeding, indicating sexual dimorphism in terms of nutrient sensing and handling (Mao et al., 2010). Conclusively, transplacental transport of lipids relies on a proper regulation at the feto-maternal interface, which is disturbed in maternal obesity.

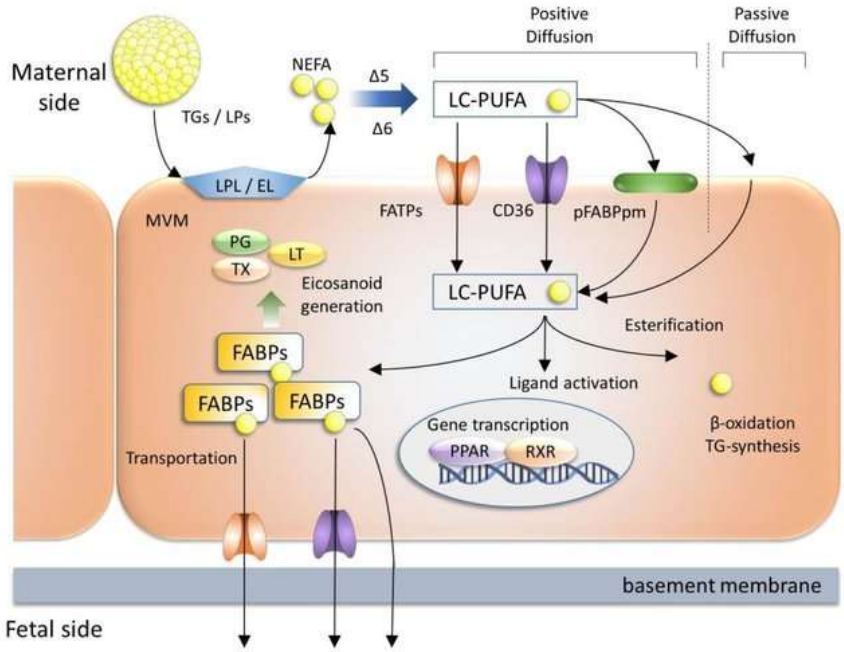

Figure 1: Schematic transplacental transport of lipids from the maternal to the fetal circulation across the placenta.
At the maternal side, lipoproteins (LP) and triglycerides are hydrolysed to produced non-esterified FAs (NEFA). They can be either transported actively by lipid transporters FATP and CD36 or passively diffuse. Within the placental cell, they can be re-esterified, act on nuclear receptors or become eicosanoids in case of PUFAs. However, most of the lipids are later secreted into the fetal circulation. Figure adapted from Islam et al. (2016).

2. Circulating parameters involved in systemic energy metabolism

The circulating metabolome plays a crucial role in altering the cell fate of different tissues during the pathogenesis of overweight. As mentioned above (see chapter 1.3), overweight is associated with a chronic low-grade inflammation. The crosstalk between nutritional status and immune function during pregnancy will be the main focus of the following chapters.

2.1 Leptin

Leptin is a key player in the regulation of appetite, systemic energy homeostasis and female reproductivity. Its role was firstly described in 1950, when mice lacking the *ob* gene (*ob/ob*), encoding leptin, were discovered (*ob* for ***ob**ese*). These mice were characterized by hyperphagia, an obese phenotype and infertility (Ingalls et al., 1996; Malik et al., 2001). Notably, the latter could be restored by intraperitoneal injection of leptin before and during gestation (Malik et al., 2001). It is expressed and secreted by adipocytes and the circulating concentrations are

proportional to body fat in non-pregnant humans (Flier, 1997). Importantly, leptin is considered to be rather related to fat mass than secreted in response to high caloric intake (Kelesidis et al., 2010). Secretion of leptin serves as a negative feedback loop to the brain to decrease food intake and increase energy expenditure, being one of the most prominent examples for adipose tissue communication with other organs (Campfield et al., 1995).

The signaling capacity of leptin is mainly conferred through the leptin receptor (LepR), more precisely through its long isoform LepRb. Mice lacking the *db* gene (for ***d**ia**b**etes*), encoding LepR, are phenotypically similar to *ob/ob* mice and are characterized by hyperphagia, obesity and infertility (Kelesidis et al., 2010). This striking phenotype was also observed in humans. Hence, mutations of LEPR are associated with hyperphagia, severe early-onset obesity and delayed puberty (Farooqi et al., 2007; Nunziata et al., 2019). Binding of leptin to LepR induces several intracellular signaling cascades, including JAK2/STAT3, IRS/PI3K and AMPK/ACC (H. K. Park & Ahima, 2014).

LepR is expressed in a variety of tissues and cell types, including the brain (mainly hypothalamus), the placenta and immune cells (Ebenbichler et al., 2002; G. H. Lee et al., 1996; Lord et al., 1998). Besides acting on the adipose tissue-brain-axis, leptin also possesses autocrine functions on adipocytes without involvement of the hypothalamus. Hence, it reduces *de novo* lipogenesis (DNL, discussed in Part A, chapter 3.1) while promoting lipolytic activity of adipocytes (Buettner et al., 2008; Shimabukuro et al., 1997; M. Y. Wang et al., 1999). In vitro experiments showed that this effect was mediated by inhibition of acetyl-CoA carboxylase (ACC) in adipocytes, thus underscoring endocrine functions of leptin in an autocrine fashion (Bai et al., 1996). As mentioned above, LepR is also expressed in the placenta(Ebenbichler et al., 2002), suggesting that leptin might be influencing endocrine functions and energy homeostasis during pregnancy. Consistently, leptin regulates the expression of placental macronutrient transporters, such as amino acid transport (von Versen-Höynck et al., 2009) or fatty acid translocase CD36 (Mousiolis et al., 2012). Of note, the placenta but not increasing maternal fat mass is the major source of increasing leptin concentrations during physiological pregnancies (Lin, 1999). Its autocrine functions include promotion of proliferation and reduction of apoptosis in trophoblasts

(Magariños et al., 2007). With regard to immunity, leptin promotes the expression of pro-inflammatory cytokines interleukin-1, interleukin-12 and tumor-necrosis factor-α (TNFα) (Carbone et al., 2012; Paz-Filho et al., 2012). The induction of interferon-γ (IFN-γ) and TNFα in memory T cells and the subsequent promotion of T helper (Th)1 cell differentiation underscore the pro-inflammatory effects of leptin (Procaccini et al., 2012). Hence, leptin is an important cytokine for the communication between nutrient availability in adipose tissue depots and placental macronutrient transport as well as immune status.

2.2 Insulin

Insulin is an anabolic hormone that is both produced and secreted by beta cells in the pancreas (Cerf, 2013). In response to high levels of systemic glucose, e.g. after a meal, insulin is secreted to act on peripheral tissues to promote glucose disposal from the circulation and stimulate lipid synthesis from glucose (see *de novo* lipogenesis in chapter 3.1) (Cerf, 2013; Young & Zechner, 2013). Insulin mainly conveys its signaling capacity through binding to the α subunit of the insulin receptor, thus leafding to autophosphorylation of the intracellular β subunit (Kasuga et al., 1982; Ullrich & Schlessinger, 1990). Following several phosphorylation events, the phosphatidylinositol 3-kinase/protein kinase B signaling pathway is activated, including inactivation of tuberous sclerosis complex 2 (Tsc2) by the serine-threonine kinase protein kinase B (PKB, also known as Akt) (Potter et al., 2002). The inactivation of Tsc2, a negative regulator of mammalian target of rapamycin complex 1 (mTORC1), facilitates the activation of mTORC1, a key player in insulin signaling (J. Huang & Manning, 2009). Conclusively, mTORC1 signaling leads to translocation of GLUT4, thus promoting glucose uptake into the cell (S. Huang & Czech, 2007). Another anabolic effect of mTORC1 is mediated through the suppression of adipose triglyceride lipase (ATGL) transcription and promoting intracellular lipid deposition (Chakrabarti et al., 2013). Other targets of mTORC1 include key players in lipid metabolism, e.g. LPL for the breakdown of very low-density lipoprotein (VLDL) particles (Paolella et al., 2020). Notably, kidney transplant recipients that received mTORC1 inhibitors displayed dyslipidemia, as indicated by hypertriglyceridemia, hypercholesterolemia and subsequent higher levels of LDL (Kasiske et al., 2008).

Consistently, the anabolic role of mTORC1 in pathophysiological conditions was demonstrated by adipocyte-specific knockout of regulatory-associated protein of mTOR (raptor), which is crucial for mTORC1 function. On control chow diet as well as high-fat diet (HFD), these mice gained less weight compared to control mice possessing functional adipocytic raptor. This weight gain phenotype was traced back to smaller adipose tissue depots and less hypertrophic adipocytes compared to control mice (Polak et al., 2008). As mTORC1 is promoting the disposition of especially carbohydrates and lipids, mTORC1 is a central mediator of insulin-mediated anabolism in a variety of tissues.

However, in pathological conditions such as overweight and obesity-associated type 2 diabetes, peripheral tissues exhibit insulin resistance and chronical low-grade inflammation. As described in chapter 1.3 (above), dysfunctional, insulin resistant AT secretes inflammatory cytokines such as TNFα, which promotes immune cell infiltration and further exacerbates insulin resistance (Pekala et al., 1983). However, heathy pregnancies are characterized by a peripheral, physiological insulin resistance, thus deviating from pathological insulin resistance during metabolic disorders. Whereas insulin sensitivity is increased during early gestation to promote glucose uptake into maternal tissues, late pregnancy is characterized by peripheral insulin resistance to ensure glucose availability for the growing fetus (Sonagra et al., 2014). Hence, the maternal pancreas produces and secretes increasing amounts of insulin to ensure adequate distribution of circulating glucose (Catalano et al., 1998). However, pre-gestational insulin resistance, e.g. in women with type 2 diabetes mellitus or obesity, is considered as a risk factor for disturbances of the favorable physiological insulin resistance and subsequent obstetric complications such as preterm birth, preeclampsia, or macrosomia (Catalano & Ehrenberg, 2006; Ehrenberg et al., 2004; Ringholm et al., 2019). Consequently, insulin is a key hormone to regulate systemic maternal energy homeostasis in order to promote lipid mobilization for transplacental transport.

2.3 Growth/differentiation factor 15

Growth/differentiation factor 15 (GDF15), a member of the transforming growth factor-β (TGF-β) superfamily, is considered as a promising therapeutic target in managing several metabolic disorders (reviewed in Rochette et al., 2020). Since

GDF15 was initially identified during a screen of the secretome from activated macrophages, it was originally named macrophage inhibitory cytokine 1 (MIC-1). In turn, the pretreatment with GDF15 suppresses macrophage activation (Bootcov et al., 1997). Thus, GDF15 has been implicated as a biomarker in a diverse range of diseases, including several types of cancer, cardiovascular health, and obesity (reviewed in Corre et al., 2013; Sarkar et al., 2020). The primary receptor of GDF15 in the regulation of body weight and appetite is GDNF family receptor α-like (GFRAL), which is mainly expressed in the brain (L. Yang et al., 2017). Recent studies linked the GDF15-GFRAL pathway to lipid metabolism and dietary intake of lipids. Thus, circulating GDF15 increased in response to medium-chain FAs, but not long-chain FAs (i.e. oleate) when fed an obesogenic diet (Kanta et al., 2023). Consistently, obese mice displayed higher circulating GDF15, reduced food intake and body mass after linoleic acid (LA) gavage; however, the effect on food intake and body weight was blunted in GFRAL deficiency (D. Wang et al., 2024). Of note, isocaloric treatment with glucose was ineffective in the reduction of food intake and or body mass, underscoring a pivotal role for GDF15 and its receptor GFRAL in lipid metabolism (D. Wang et al., 2024). However, apart from regulating appetite and subsequent food intake, the GDF15-GFRAL axis is also involved in increasing fatty acid oxidation in skeletal muscle by modulating β-adrenergic tonus (D. Wang et al., 2023).

Notably, compared to other tissues, GDF15 is highly expressed in the placenta, especially in syncytiotrophoblasts, which is in line with the increasing gestational concentrations of GDF15 observed in many studies (Fairlie et al., 1999; Lawton et al., 1997; Marjono et al., 2003). Consistently, low levels of circulating GDF15 were detected in patients with unexplained recurrent pregnancy loss (Lyu et al., 2023), suggesting a role of GDF15 in pregnancy initiation and/or maintenance. In spite of that, literature is controversial about the association between serum GDF15 and gestational complications such as preeclampsia (Q. Chen et al., 2016; Marjono et al., 2003; Temel Yuksel et al., 2018). In summary, GDF15 is highly increased during gestation and implicated in the immunometabolic crosstalk with special attention to lipid metabolism.

2.4 TREM2

Triggering receptor expressed on myeloid cells 2 (TREM2) is predominantly expressed by macrophages and was originally discovered in Nasu-Hakola, which is a disease with features of dementia and bone cysts (Bouchon et al., 2000; Paloneva et al., 2002). The pathology of Nasu-Hakola disease was mainly attributed to the decreased phagocytic capacity of TREM2 deficient microglial cells, thus leading to insufficient clearance of apoptotic neuronal cells (Takahashi et al., 2005). Hence, several studies linked TREM2 to neurodegenerative disorders, including Alzheimer's disease, Parkinson´s disease and multiple sclerosis (reviewed in Filipello et al., 2022). Moreover, macrophages are important determinants of adipose tissue remodeling during the development of obesity due to their capacity to remove dead adipocytes (reviewed in McNelis & Olefsky, 2014). Notably, TREM2 has the capacity to bind several lipids, including phosphatidylserine, PC, PE, phosphatidylinositol and SM (Cannon et al., 2012; Y. Wang, Cella, et al., 2015). Since TREM2 is a phagocytic marker of macrophages with the capacity to bind lipids, its role in lipid metabolism and metabolic disorders has been investigated intensively. With regard to this, it is worth mentioning that macrophage infiltration into AT was observed at the onset of obesity (Weisberg et al., 2003), underscoring the role of macrophages in AT remodeling. Consequently, TREM2 was described as a pivotal factor of lipid associated macrophages (LAMs), characterized by a phagocytic and lipid metabolic gene signature, to sense hyperlipidemia and remove hypertrophic adipocytes (Jaitin et al., 2019). A recent study revealed that TREM2 expression in adipose tissue correlated with BMI, whereas this association was only statistically significant in male participants (Reich et al., 2023). Consistently, single nucleotide polymorphism in the TREM2 gene was associated with increased BMI (Reich et al., 2023). Moreover, TREM2 was found to be elevated in the liver of patients with NAFLD, as demonstrated by increased circulating and hepatic TREM2. Importantly, TREM2 correlates with typical characteristics observed in patients with NAFLD, i.e. plasma alanine transaminase (ALT) levels, hepatic fibrotic markers and TAG content (Hendrikx et al., 2022; Indira Chandran et al., 2023; Liebold et al., 2023).

Although pregnancy is a state which demands a high capacity of tissue remodeling particularly in the placenta (reviewed in Faas & De Vos, 2018), TREM2 during gestation is largely unexplored. However, the high abundance of TREM2-

positive macrophages in the placenta compared to many other tissues (Deczkowska et al., 2020), together with the function of TREM2 in lipid metabolism discussed above, suggest a role in placental tissue remodeling and lipid processing. Consistently, human decidual macrophages express high levels of TREM2 compared to circulating macrophages (Gustafsson et al., 2008). Moreover, in a mouse model of fetal loss, the transfer of regulatory T cells reduced fetal loss and was associated with increased uterine macrophages, characterized by high gene expression of Trem2 among others (Lewis et al., 2023). Importantly, this gene signature of decidual macrophages seems to be conserved between mice and human (Lewis et al., 2023). To sum up, the role of TREM2 in macrophages seems to be particularly important in processes that demand a high capacity of tissue remodeling and clearance of lipids.

3. Lipids as a diverse class of molecules

During the last decades, the idea/dogma of lipids as simple molecules in energy supply was replaced by a more diverse understanding of the role of lipids in many cellular and systemic processes. Historically, lipids were considered primarily as energy source during prolonged starvation, thus ensuring survival in times of nutrient unavailability. As biochemical and molecular techniques as well as knowledge advanced, the scientific community created a more detailed picture on the range of lipid classes and individual species in a large variety of processes. This diversity reaches from serving as a crucial part of cell membranes determining membrane fluidity (e.g. in brain) and precursor for hormone synthesis (e.g. cholesterol) and possessing bioactivity to alter intracellular responses and cell fate (J. Park et al., 2021; Wymann & Schneiter, 2008). Owing to the heterogeneity of lipids and the thereby resulting characteristics, the following chapters clarify the biology of most important lipid classes analysed in this study. Additionally, their role in pathogenic processes, overweight and pregnancy will be elucidated.

3.1 Fatty acids, di- and triglyceride

The role of di- (DAG) and triglycerides (TAG) is mainly attributed to the canonical understanding of lipids as energy storage. Generally, both lipids possess a glycerol backbone, which in principle could be esterified to fatty acids (FAs) at three different positions. DAG is esterified to FAs at two positions, whereas TAG to three

FAs (J. Park et al., 2021). Firstly, FAs are either taken up from the diet or could be synthesised from glucose, a process called *de novo* lipogenesis (DNL). DNL mainly occurs in the liver, but extrahepatic organs, such as adipose tissue depots, could also synthesize FAs themselves from carbohydrates. High blood glucose levels trigger the activation of carbohydrate responsive element binding protein (ChREBP) and subsequent induction of enzymes involved in DNL (Eissing et al., 2013; reviewed in Y. Wang, Viscarra, et al., 2015). Major genes involved in DNL are pyruvate kinase (encoded by PKLR), fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturases (SCD), and several glucose transporters (GLUTs) (see Figure 2).

After transcriptional activation by ChREBP, the respective enzymes contribute to DNL from glucose. As a first step, glucose is converted into pyruvate in a series of enzymatic reactions during the process of glycolysis. After glycolysis, acetyl-CoA is formed from pyruvate. Next, acetyl-CoA is carboxylated by ACC to synthesize malonyl-CoA. The synthesis of FAs from malonyl-CoA is performed by FASN (reviewed in Solinas et al., 2015). The resulting saturated FAs are processed by SCD and elongases to become desaturated and elongated, respectively (Nagle et al., 2009). Ultimately, these FAs are incorporated into DAG by acyltransferases such as diacylglycerol O-acyltransferase (DGAT) to generate TAG for lipid storage. However, if energy in form of FAs is needed, the body is able to release FAs esterified to TAG and other lipid classes (i.e. cholesteryl esters or phospholipids) during lipolysis. Depending on the nature of the lipid class and the tissue, this process involves a diversity of lipases, such as hormone sensitive lipase (HSL, encoded by LIPE), phospholipase A2 (PLA2), lipoprotein lipase (LPL) or adipose triglyceride lipase (ATGL) (reviewed in Grabner et al., 2021).

Notably, FAs derived from DNL are primarily saturated or monounsaturated, and are higher in patients with obesity-associated disorders (e.g. NAFLD, type 2 diabetes mellitus) (Roumans et al., 2020). In line with that, FASN inhibition in an obesogenic mouse model promoted the uptake of polyunsaturated FAs (PUFAs), leading to subsequent increased incorporation of PUFAs into TAG (Worthmann et al., 2024).

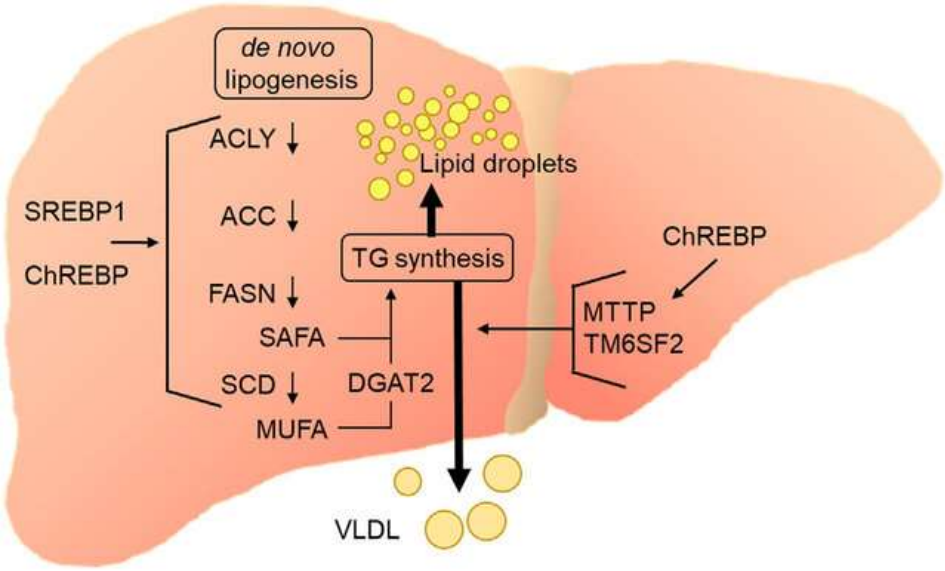

Figure 2: Hepatic *de novo* lipogenesis (DNL).
Postprandial glucose stimulates the activity of the transcription factor ChREBP, which regulates DNL. This leads to expression of enzymes involved in the stepwise synthesis of lipids from carbohydrates, i.e. ACLY, ACC, FASN and SCD. The resulting FAs are esterified to diacylglycerol (DAG) to generate triglycerol (TAG). TAG is either stored in intracellular lipid droplets or secreted in very low-density lipoproteins (VLDL) to reach peripheral tissues. Figure adapted from Heeren & Scheja (2021).

3.2 Essential and polyunsaturated fatty acids

Essential FAs are usually polyunsaturated and exclusively obtained from diet as the human body is not able to synthesise them itself. Classical sources of essential FAs are seafood, nuts, seeds, or plant oils such as olive or sunflower oil. Many of those are implicated in the so-called Mediterranean Diet, which is mainly based on vegetables, fruits and a moderate consumption of seafood and dairy products, although a unifying definition does not exist (Davis et al., 2015).

As the name indicates, essential FAs, such as omega-3 (ω-3) and -6 FAs, are crucial for membrane fluidity (Hulbert et al., 2005). This feature is important in almost all membranes, whereas the brain and immune cells have an increased demand of PUFAs for proper functioning (Harris & Baack, 2015). The essential nature for the human metabolism was demonstrated in a study comparing infant milk formulas without essential FAs or with linoleic acid (LA; an ω-6 FA). Infant formulas devoid of essential FAs caused diarrhoea and skin issues, which was reversed when switched to a formula containing LA (Hansen et al., 1958). This underscores the importance of essential FA supply from the mother even in postnatal life. Of note, especially phospholipid (PL) derived essential PUFAs are important for the fetus since the FAs are of dietary origin and the fetus lacks the capacity to synthesise them on its own

(Larqué et al., 2011). Thus, it relies on maternal PUFA provision and maternal PUFA status. This topic will be discussed more detailed in the following chapter (chapter 3.3).

The structure of essential FAs differs from their counterparts derived during mammalian DNL as they also possess double bonds at positions closer to the methyl end (see Figure 3, Nicolai et al. (2017)). Although the human body is incapable to synthesize essential FAs *de novo*, it can modify them further after dietary uptake. These processes involve the elongation and desaturation of dietary lipids to produce longer and more desaturated FAs (reviewed in Kaur et al., 2014). However, humans can desaturate FAs only at specific positions, namely position ω-9 or closer to the carboxylic end. Notably, expression of the respective mammalian Δ6-desaturase was found in tissues where more complex and desaturated FAs are needed, including brain and fetal tissues (Nakaruma et al., 2001).

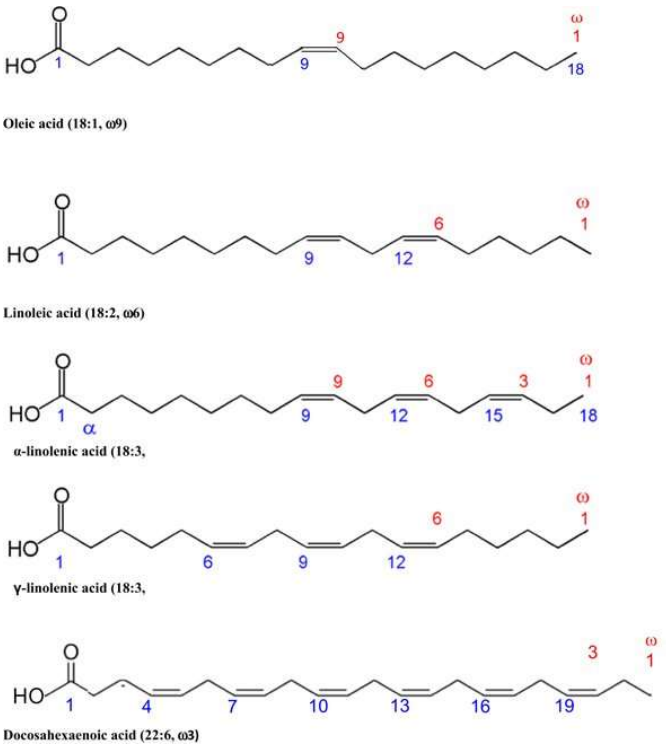

Figure 3: Representative members of non-essential and essential fatty acids (FAs).
Based on the first double from the methyl end of FAs, they are categorized into non-essential FAs, which possess double bond at position omega (ω)-9 or higher, and essential FAs (ω-6 and ω-3). Oleic acid is a non-essential FA, whereas linoleic acid, linolenic acids and docosahexanoic acid are essential FAs and polyunsaturated as they possess more than one double bond. Carbons numbers counted from the ω-terminus are highlighted in red, whereas carbons counted from the carboxylic end are highlighted in blue. Figure adapted from Nicolai et al. (2017).

3.3 Essential fatty acids during health and disease

Recent meta-analyses and systematic reviews underscore the importance of essential FAs in many pathophysiological conditions such as cardiovascular diseases and cognitive health as supplementation improved lipid parameters implicated in lipoprotein metabolism (Bernasconi et al., 2021; Sala-Vila et al., 2022; B. Z. Wei et al., 2023). Population-based studies often make usage of the Mediterranean Diet as a measure of essential FA intake. In line with studies focussing on essential FA supplementation, adherence to the Mediterranean Diet was shown to have beneficial health effects in participants with overweight and obesity, as plasma cholesterol in accordance with BMI were lowered (Lotfi et al., 2022; Meslier et al., 2020), and partially these effects could be attributed to lowered hepatic lipid content (Gepner et al., 2019). Patients with coronary heart disease that were prescribed Mediterranean Diet had less incidences of cardiovascular events (e.g. ischaemic stroke, myocardial

infarction, cardiovascular death) compared to patients on a low-fat diet during a study period of seven years (Delgado-Lista et al., 2022), underscoring the potential of dietary interventions and, presumably, essential FAs in preventing cardiovascular disease.

This is in line with a meta-analysis, demonstrating that supplementation of essential FAs during pregnancy leads to positive effects during gestation (i.e. greater gestational length, tendency for reduced pre-eclampsia) and for the offspring (i.e. measures related to neurocognitive functions and behaviour) (Middleton et al., 2018). Consistently, placental expression of the lyso-phospholipid transporter major facilitator superfamily domain-containing protein 2a (MFSD2A), which prefers docosahexaenoic acid (DHA), was lower in pregnancies complicated by gestational diabetes mellitus and correlated with cord blood DHA, suggesting a role in transplacental PUFA transport (Prieto-Sánchez et al., 2017). In line with that, placental knockdown of Mfsd2a in mice lead to decreased fetal brain weight. Additionally, the PUFA DHA content in the brain was lower compared to control mice (Powell, Barentsen, et al., 2023). Regarding ω-6 FAs, maternal obesity is associated with an altered FA profile, characterized by higher blood levels of arachidonic acid (AA) (Scifres et al., 2014). Particularly AA is associated with reproductive complications (Kikut et al., 2020; J. Li et al., 2018), which might be partially traced back to its role as a precursor for proinflammatory prostaglandins and leukotrienes (Rieger, 1985). Of note, cord blood ω-6 to ω-3 PUFA ratio was associated with offspring BMI at two and ten years of age (Standl et al., 2014).

Moreover, maternal PUFA status was linked to offspring atopic manifestations in a prospective pregnancy cohort. Thus, maternal ratio of ω-6 to ω-3 PUFAs and additionally AA were associated with the risk to develop eczema in early postnatal life, but this association disappeared until the age of seven years (Notenboom et al., 2011). However, although maternal fish oil supplementation (enriched in ω-3 FAs) results in reduced neonatal circulating IL-13 compared to the placebo group, no other measures related to inflammation and atopic manifestations were different between the groups (e.g. IgE, TNFα and several interleukins) (Dunstan et al., 2003). However, these parameters were undetectable in almost all samples to a similar extend in both experimental groups (Dunstan et al., 2003), but other trial study similarly observed

absent associations between maternal fish oil supplementation and cord blood cytokine profiles (Mozurkewich et al., 2018). Conversely, another study observed a beneficial effect of fish oil supplementation on cord blood cytokine profile in line with decreased abundance of cord blood natural killer (NK) and $CCR3^{+}CD8^{+}$ T cells; the latter might be linked to a decreased T_H2 response in the fetus (Krauss-Etschmann et al., 2008). Due to the large body of research indicating beneficial effects of PUFAs for non-pregnant individuals and particularly during pregnancy with regards to metabolic health, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends consuming at least 200 mg DHA per day (Koletzko et al., 2007). To sum up, PUFAs are essential for various processes within the body and gestational insufficiency is associated with offspring overweight, parameters of neurocognitive behaviour and in part atopic disorders.

3.4 Glycerophospholipids

Generally, glycerophospholipids (PLs) are a diverse class of lipids, as they can differ by their head group or their number of acyl chains. Generally, they can be synthesised in the Kennedy pathway from choline or ethanolamine, which are added stepwise to DAG (Kennedy & Weiss, 1956; Saito et al., 2022). Although there are existing many PLs, this work focusses on the following four classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE) and their respective derivates lyso-PC (LPC) and lyso-PE (LPE), which are lacking one of the originally two acyl chains due to hydrolysis by different (phospho-)lipases. For instance, PLA2 mainly hydrolyses membrane PC into LPC and contributes to release of pro-inflammatory arachidonic acid, which is used for prostaglandin syntheses (Murakami et al., 1996). LPC could be re-esterified by lysophosphatidylcholine acyltransferase (LPCAT)(reviewed in Shindou et al., 2009).

PLs are a diverse class of lipids. Structurally, they are of amphipathic nature. They are built up with a hydrophilic headgroup and a hydrophobic tail, which allows PLs to build lipid double membranes in aquatic solutions (reviewed in Farooqui et al., 2000). This polar feature makes PLs an important component of cellular membranes. Moreover, they are crucial components of very low-density lipoprotein (VLDL), and insufficient phosphatidylcholine (PC) synthesis is linked to decreased hepatic VLDL secretion (Z. Yao & Vance, 1988). A recent study showed that the placenta has a

high capacity to remodel PLs, which leads to the incorporation of PUFAs and probably shuttling to the fetus (Powell et al., 2024), thus underscoring the role of PLs as a transport system in many processes. The characteristic to shield and transport hydrophobic substances is also of interest for pharmacology, as PLs are used as carriers in several drug delivery systems (J. Li et al., 2015).

Within the PLs, another distinct subgroup exists. These ether-PLs are characterized by either an ether or vinyl-ether linkage between the *sn*-1 position of the glycerol backbone and the fatty acid. At the *sn*-2 position, they usually possess a PUFA (reviewed in Braverman & Moser, 2012; Farooqui et al., 2000). As other PLs, ether-PLs are an important part of cell membranes and make up 20% of the total PL pool and in the brain around 85% of the PE pool (Han et al., 2001). Due to their distinct structural properties, they might be involved in determining membrane curvature and dynamics, thus potentially contributing to membrane fission and fusion in exo- and endocytosis, respectively (Koivuniemi, 2017). Consistently, plasmalogens were shown to supress microglial endocytosis through inhibition of toll-like receptor 4 (TLR4) (Ali et al., 2019). Moreover, especially the vinyl-ether bond of so-called plasmalogens was thought to convey antioxidant capacities, thus presumably playing a role in the protection of cells from reactive oxygen species (ROS) and contributing to the reduction of oxidative stress (reviewed in Braverman & Moser, 2012). Additionally, ether phospholipids were negatively associated with BMI, subcutaneous fat, and insulin resistance in a monozygotic twin study (Pietiläinen et al., 2007). Conclusively, PLs are a major vehicle for the transplacental transport of essential FAs and might be implicated in ameliorating oxidative stress.

3.5 Sphingolipids

In contrast to glycerophospholipids, sphingolipids contain a sphingosine backbone with a monounsaturated 18-carbon chain and an amine group. The amine group is connected to a fatty acid, which is variable in length and saturation. The head group of sphingolipids is critical for the discrimination of individual sphingolipid subgroups and is linked to either phosphorylated headgroups (sphingomyelin), sugar (complex glycosphingolipids) or without any headgroup (ceramide) (Hannun & Obeid, 2017).

The members of this complex and diverse lipid class can be synthesized in three different pathways to produce ceramide (CER), which can be further converted into more complex glycosphingolipids (i.e. hexosylceramide (HCER) and lactosylceramide (LCER)) (see Figure 4). Notably, most of the reactions within the sphingolipid synthesis pathways are reversible. The *de novo* synthesis pathway of sphingolipids takes place in the endoplasmic reticulum (ER) and starts with palmitoyl-CoA to produce sphinganine, which is used by ceramide synthase (CerS) to synthesize dihydroceramide (DCER). DCER is then desaturated to produce CER. In the second pathway, sphingomyelin (SM) is catabolized by sphingomyelinase to produce CER and additionally choline as a side product. The third option is the salvage pathway, which starts with the dephosphorylation of sphingosine-1-phosphate. The subsequent sphingosine is esterified to an FA by CerS to produce CER. Notably, depending on the FA that is incorporated and the cell type in which synthesis takes place, six different CerS enzymes found in mammalians are involved and they possess distinct FA-specificities and expression patterns (reviewed in Hannun & Obeid, 2017; Levy & Futerman, 2010; Simon et al., 2019). CerS1, which prefers an acyl chain-length of C18, is mainly expressed in the brain, whereas CerS2 (found in kidney and liver) and CerS3 (testis, skin) prefer very long-chain FAs with acyl chain-length of C20 to C26 and C22 to C26, respectively (Levy & Futerman, 2010).

In line with the diverse expression pattern of CerS enzymes, abnormal sphingolipid levels in the circulation and several tissues have been associated to a variety of disorders. As such, sphingolipid metabolism is disrupted in patients with non-alcoholic steatohepatitis (NASH) (Apostolopoulou et al., 2018). Inhibition of *de novo* ceramide synthesis was shown to have beneficial effects in metabolic disorders, including body weight loss and improvement of insulin sensitivity (reviewed in Simon et al., 2019). However, sphingolipids were also shown to act as signalling molecules in various processes. As such, long chain sphingolipids (C16, C18, C20) were implicated in inhibition of cell proliferation and promotion of apoptosis, whereas very long chain fatty acid (e.g. C24) containing sphingolipids had opposite effects in breast cancer and colon cancer cell lines as demonstrated by overexpression of the respective CerS enzymes (Hartmann et al., 2012). In adipocytes, sphingolipids modulate the expression of glucose transporters independently of insulin signalling

(C. N. Wang et al., 1998). Notably, the CER derivate ceramide 1-phosphate directly acts on phospholipase A2 to promote arachidonic acid (AA) release and consequently contributes to prostaglandin synthesis and inflammatory signalling (Pettus et al., 2004). Additionally, sphingolipid signalling plays a key role in placental vascularization and embryonic survival as knockout of sphingosine-1-phosphate receptor is lethal (Y. Liu et al., 2000). Besides, sphingolipids increase with the start of gestation, suggesting their importance in physiological pregnancy (Enthoven et al., 2023). However, elevated first trimester levels of SM species are associated with preeclampsia (Dobierzewska et al., 2017). Similarly, SM esterified to palmitic acid was higher in pregnant women with overweight or obesity compared to lean mothers, whereas the abundancy of several CER species was lower (León-Aguilar et al., 2019). In summary, sphingolipids are a diverse and bioactive class of lipids implicated in the pathogenesis of metabolic disorders and female infertility.

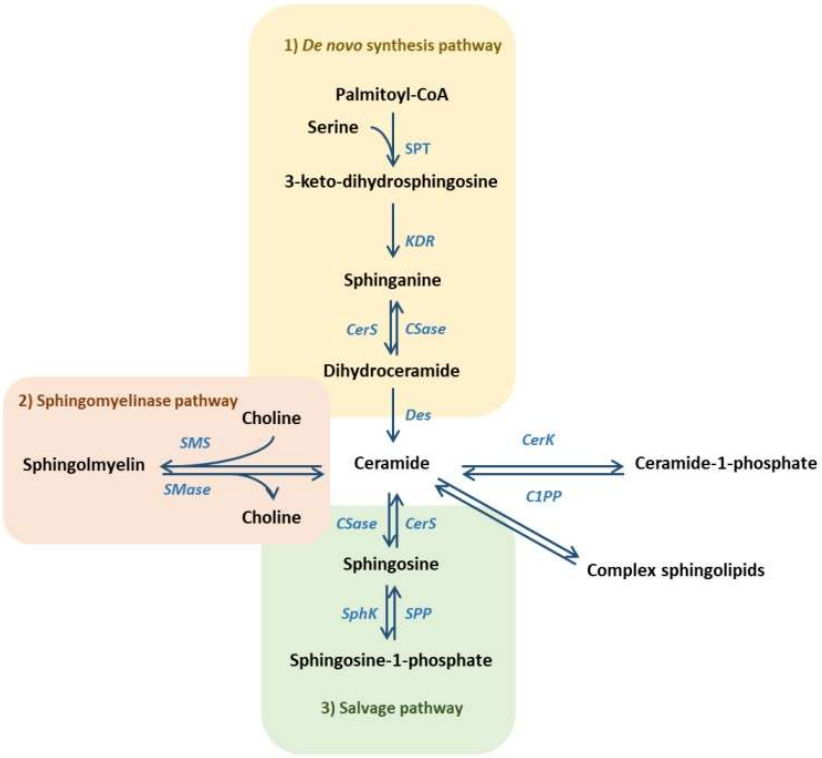

Figure 4: Sphingolipid synthesis pathways.
Ceramide can be produced either 1) in the *de novo* synthesis pathway from palmitoyl-CoA and serine, 2) from sphingomyelin, or 3) in the salvage pathway. SPT = serine palmitoyltransferase; KDR = 3-keto-dihydrosphingosine reductase; CerS = ceramide synthase; CSase = ceramidase; Des = desaturase; SphK = sphingosine kinase; SPP = S1P phosphatase; SMase = sphingomyelinase; SMS = sphingomyelin synthase; CerK = ceramide kinase; C1PP = ceramide-1-phosphate phosphatase. Figure adapted from Simon et al. (2019).

4. Bile acids as cholesterol derivates

Cholesterol is an amphipathic molecule with important roles in membrane permeability. However, it also serves as a precursor for the synthesis steroid hormones and bile acids (BAs) (Schade et al., 2020). Especially the conversion of insoluble, non-polar cholesterol into water-soluble, amphipathic BAs is the major pathway for cholesterol metabolization, thus being a substantial contributor to cholesterol clearance (Russell, 2003). The *de novo* synthesis of BAs mainly occurs in the liver and involves a stepwise oxidation and conjugation cascade (see following chapter).

4.1 BA synthesis

The synthesis of BAs from cholesterol occurs mainly in the liver and involves a series of hydroxylation steps. In principle there are two pathways for the *de novo* synthesis of BAs: the classical/neutral and the alternative/acidic pathway. However, 75% of the newly synthesised BAs are derived from the classical pathway, with cholic acid (CA) being the main and chenodeoxycholic acid (CDCA) being the minor (Thomas et al., 2008). The alternative pathway and its main product CDCA account for the remaining ca. 25% as knock out mouse models for the respective enzymes suggest (Russell, 2003).

In the classical pathway, cholesterol-7α-hydroxylase (encoded by CYP7A1) is the rate limiting enzyme and responsible for the hydroxylation of cholesterol at the C-7 position (Russell, 2003; Thomas et al., 2008). Notably, humans with a mutation in the CYP7A1 gene were associated with hypercholesterolaemia and a subsequent increase in hepatic cholesterol content (Pullinger et al., 2002). The last step in the classical pathway is performed by sterol 12α-hydroxylase (CYP8B1) to generate CA. The alternative pathway is initiated by sterol 27-hydroxylase (CYP27A1), which hydroxylates cholesterol preferentially at position C27, thus generating 27α-hydroxycholesterol (Lund et al., 1993). An important step in the alternative pathway is the hydroxylation of 27α-hydroxycholesterol at C7 by oxysterol-7α-hydroxylase (CYP7B1), which leads to the synthesis of CDCA (Russell, 2003).

The end products of the *de novo* BA synthesis pathways CA and CDCA are so-called primary BAs (Russell, 2003). After synthesis in the classical or alternative pathway, both CA and CDCA are conjugated to glycine and to a lesser extent to

taurine in adult humans to reduce their increase their solubility (reviewed in Evangelakos et al., 2021). Notably, biliary CA and CDCA in human fetuses are primarily conjugated to taurine, but this difference in the conjugation pattern disappears and resembles the one of adult humans during the first year of life (Encrantz & Sjövall, 1959; Setchell et al., 1988). These conjugated BAs are stored in the gallbladder and can be secreted upon food intake into the intestinal tract. There, BAs facilitate the absorption and uptake of water insoluble molecules such as dietary lipids and lipophilic vitamins (Thomas et al., 2008). However, in the colon, BAs are deconjugated by microbes expressing bile salt hydrolase (BSH) and further modify them via e.g. dehydroxylation, isopropylation and oxidation to produce secondary BAs (reviewed in R. Yang & Qian, 2022).

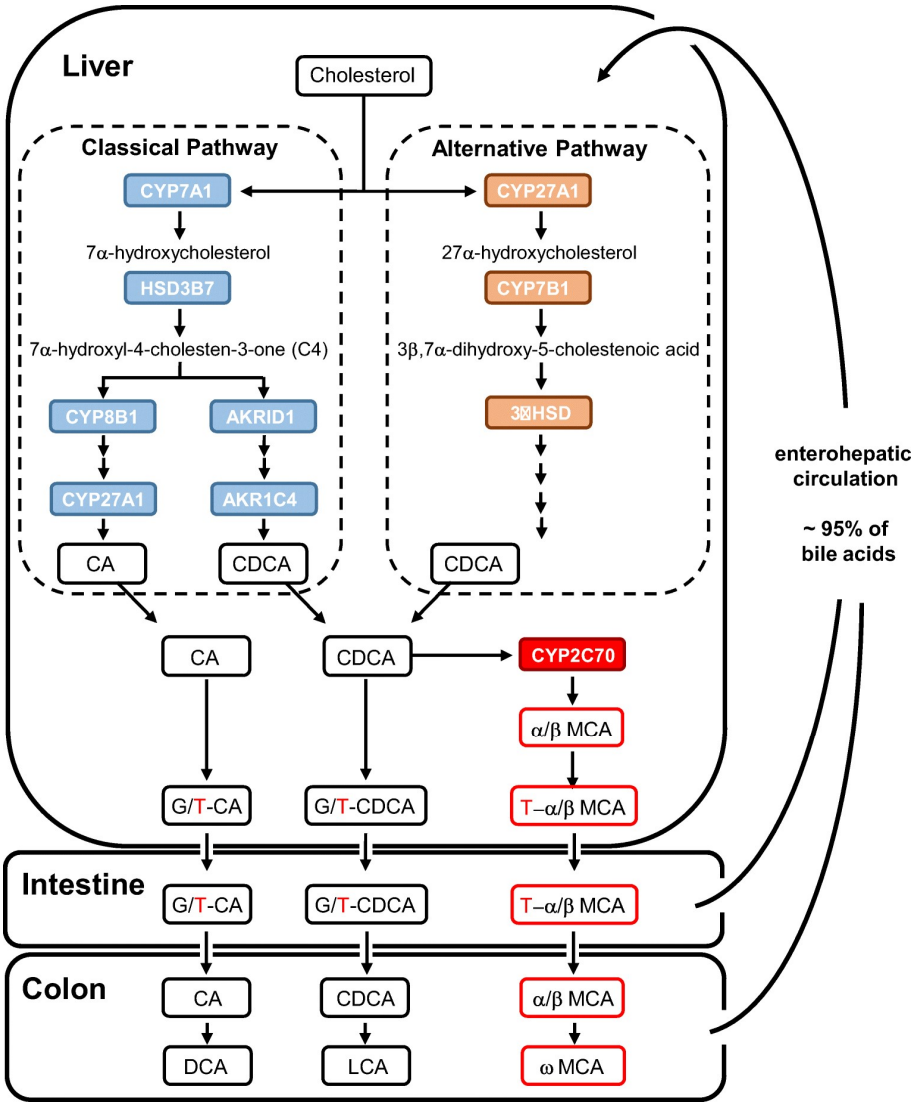

Figure 5: Synthesis of bile acids from cholesterol in the liver and enterohepatic circulation.
In the classical pathway of bile acid (BA) synthesis, cholesterol is used by CYP7A1 as the initial step to synthesize either cholic acid (CA) or chenodeoxycholic acid (CDCA). The alternative pathway starts with CYP27A1 and involves participation of CYP7B1 to produce CDCA. In mice, CDCA is further hydroxylated by CYP2C70 to produce muricholic acid (MCA). These *de novo* synthesized BAs are conjugated to either glycine or taurine to increase their solubility. After a meal, these conjugated BAs are secreted into the intestine to facilitate dietary uptake of lipophilic nutrients. Microbiota in the colon first deconjugate and afterwards modify BAs to generate secondary BAs (e.g. deoxycholic acid (DCA), lithocholic acid (LCA)). 95% of the colonic BAs are reabsorbed and transported back to the liver through the enterohepatic circulation. Enzymes of the classical pathway are highlighted in blue, enzymes from the alternative pathway in orange and the murine specific enzyme CYP2C70 in red. Murine specific BAs or conjugation patterns (i.e. taurine) are highlighted in red. G = glycine; T = taurine. Figure adapted from Evangelakos et al. (2021).

Especially the site and position of hydroxylation is a major determinant of physicochemical properties and, thus, the hydrophobicity of BAs (Carey, 1984; Lefebvre et al., 2009; Thomas et al., 2008). Generally, the hydrophobicity is decreasing from lithocholic acid (LCA) over CDCA, CA and ursodeoxycholic acid (UDCA) to murine-specific muricholic acids (MCA). Notably, hydrophobic BAs are more toxic and have membrane disrupting properties, thus being cytotoxic and antibiotic. The hydrophobicity determines bile flow and the capacity to solubilize lipophilic nutrients. Hydrophilic BAs in turn are less toxic and promote bile flow (Thomas et al., 2008). Consistently, the hydrophobicity of BAs determines their potential to induce apoptotic processes within the cell. This could be either mechanically/chemically to due membrane disruption or indirectly via inducing mitochondrial dysfunction or signalling pathways within the cell (Abrigo et al., 2022; reviewed in S. Wei et al., 2020). However, the capacity of BAs to induce apoptosis also depends on BA concentration or conjugation (Perez & Britz, 2009; Thomas et al., 2008).

Besides hydrophobicity of the BA pool, it is noteworthy that the total amount of BAs in the circulation needs to be regulated accurately during pregnancy. In pathological conditions such as intrahepatic cholestasis of pregnancy (ICP), mothers display extremely high concentrations of BAs in the circulation (Heikkinen et al., 1981). This affects the maternal and placental capacity to clear the fetal circulation from harmful substances, ultimately leading to impaired maternal glucose tolerance, increased oxidative stress and risk of preterm birth (C. Liu et al., 2020; Perez et al., 2006). Notably, most of the adverse health outcomes in ICP can be reversed by administration of the hydrophilic BA UDCA (Perez et al., 2006).

Although the capacity of the fetal liver to synthesize BAs *de novo* from cholesterol is limited, the fetus performs several BA modifications endogenously (Kimura et al., 1994; Setchell et al., 1988). Notably, high levels of BAs at birth are potentially harmful, as neonatal enterohepatic circulation is immature (Balistreri et al., 1983). As the mother excretes excess BAs through feces or urine, fetuses developed mechanisms to detoxify and eliminate toxic BA levels. These mechanisms are already existent in the first trimester of pregnancy as fetuses hydroxylate and sulphate BAs extensively by that time (Colombo et al., 1987; Setchell et al., 1988;

Wood et al., 2017). Notably, these modifications are generally implied as detoxification mechanisms to reduce hydrophobicity (Alnouti, 2009; Kastrinou Lampou et al., 2023). Notably, this distinct fetal BA metabolism leads to the production of “unusual” BAs for adult individuals. Usually, these BAs are undetectable in adult individuals, but abundant in the urine of pregnant women and even more pronounced in newborns, thus being particularly “newborn-specific” (Seki et al., 2011; W. X. Wang et al., 2020). Moreover, elevated abundance of these unusual BAs in the urine of pregnant women compared to non-pregnant women suggests a reversed transplacental transport of these BAs (Seki et al., 2011). This demonstrates that the BA metabolism is highly variable during human life and undergoes maturation, especially when comparing maternal to fetal BA metabolism.

4.2 BA signalling

Besides their canonical function in the absorption of dietary lipids, BAs are recently recognized as bioactive molecules that contribute to the regulation of several metabolic and immunological processes. The signalling capacity is predominantly mediated via the two main BA receptors TGR5 and FXR (Thomas et al., 2008). Takeda G protein-coupled receptor 5 (TGR5) is a G-protein coupled receptor at the membrane which is primarily activated by hydrophobic BAs. It is expressed in a variety of tissues including different hepatic cell types, enterocytes, brain, placental syncytiotrophoblasts and adipose depots (Kawamata et al., 2003). Notably, the supplementation of CDCA in humans leads to higher energy expenditure, which was linked to TGR5 signalling in brown adipocytes (Broeders et al., 2015). TGR5 activation in macrophages leads to reduced secretion of inflammatory cytokines such as TNFα (Pols et al., 2011), thus connecting metabolic status with immune function.

The nuclear BA receptor farnesoid X receptor (FXR) possesses a distinct expression pattern, as it is highly expressed in liver, gut, adrenal gland and to a lesser extent in adipose tissue as well as heart (Zhang et al., 2003). In general, hydrophobic BAs such as CDCA, DCA and LCA are agonistic, whereas hydrophilic BAs such as muricholic acids or UDCA are antagonistic for FXR signalling (Lew et al., 2004; Makishima et al., 1999; Parks et al., 1999; Sayin et al., 2013). Antagonism of FXR leads to induction of small heterodimer partner (SHP) and subsequent suppression of *de novo* BA synthesis through Cyp7a1 (Lu et al., 2000). In the

intestine, FXR activation leads to intestinal fibroblast growth factor (FGF) 15/19 expression and secretion (FGF15 is the murine orthologue of human FGF19). FGF15/19 reaches the liver via the portal vein and binds to hepatic FGF receptor 4, which lowers Cyp7a1 expression and, thus, serves as feedback for *de novo* BA synthesis (Inagaki et al., 2005). Apart from synthesis of BAs, FXR signalling was also implied in the regulation of DNL as it decreased hepatic SCD1, acetyl-CoA synthetase and sterol regulatory element-binding protein 1c (SREBP-1c) gene expression, thus regulating hepatic lipid content (Watanabe et al., 2004).

In brown fat and skeletal muscle, BAs induce the expression of type 2 iodothyronine deiodinase (DIO2), which contributes to a higher metabolic rate (Watanabe et al., 2006). Recently, the polyhydroxylated and hydrophilic BA hyocholic acid (HCA) was discovered to be a marker of metabolic syndrome in humans (Zheng, Chen, Zhao, et al., 2021). HCA is an unusual BA for humans, whereas it is highly abundant in pigs, which are known for their remarkable resistance to development diabetic conditions (Gerstein & Waltman, 2006; Zheng, Chen, Jiang, et al., 2021). Notably, HCA possesses the unique capacity to mediate signalling effects through the two main BA receptors in enterocytes, thus regulating systemic glucose homeostasis (Zheng, Chen, Jiang, et al., 2021).

Altogether, this highlights that BAs have a high capacity to alter metabolic processes in a variety of tissues, thus contributing to systemic energy homeostasis.

Part B: Aims of the study

Generally, overweight and obesity are characterized by disturbances of the endogenous lipid metabolism and immune balance. Healthy pregnancies have long been recognized as the basic for offspring vitality. Pregnancy itself is a state of tightly regulated metabolic and immunological adaptions to maintain pregnancy and ensure maternal and offspring health. However, this regulation is disturbed during maternal overweight and obesity as characterized by an altered fatty acid profile, hyperlipidemia and a pro-inflammatory environment. Healthy placentas possess a certain buffering capacity to selectively supply lipids to the fetus, but this transplacental transport is defected during overweight.

Canonically, lipids were thought to aid as caloric reserves during times of starvation or energy demanding conditions including exercise or nourishing a growing fetus. More recently, lipids were implicated in the etiology of metabolic disorders including insulin resistance, non-alcoholic fatty liver disease and cardiovascular disease. In the context of female reproduction, disturbances of the circulating lipidome are strongly linked to pregnancy loss, preterm birth, or preeclampsia.

This study aims to provide a better understanding of how maternal overweight and the underlying metabolic and particularly lipidomic changes during pregnancy might contribute to the inheritance of overweight and associated disorders. Therefore, this study elucidates the impact of maternal overweight on both maternal and fetal lipid metabolism. The findings should create a basis for future research to break the transgenerational inheritance of overweight.

Part C: Material and Methods

1. PRINCE cohort

Study participants of the prenatal identification of children´s health (PRINCE) study were recruited at the Klinik für Geburtshilfe und Pränatalmedizin, University Medical Center Hamburg. All participants gave informed consent. The study protocol was approved by the ethics committee (Ethik-Kommission der Hamburger Ärztekammer, Approval No: PV3694). Women older than 18 years with a healthy singleton at gestational week 12-14 were included. However, women with medical history, drug usage (alcohol, tobacco), chronic medication or pregnancies conceived after assisted reproductive technologies were excluded. The remaining participants were invited for maternal blood draw, a detailed ultrasound examination, psychometric and lifestyle questionnaires at each trimester (gestational week 12 to 14, 24 to 26, and 34 to 36). The body mass index (BMI) was calculated as weight in kilogram divided by the square of the height in meter. The BMI at the first examination was used to categorize the participants into a normal BMI group (BMI <25 kg/m²) and women with overweight (≥25 kg/m²). Groups were matched by age, parity, gestational weight gain and fetal sex to minimize the effect of potential confounders (Table 1).

Table 1: Demographics of study participants.

	Normal BMI (<25 kg/m², *n* = 86)	**Overweight (BMI ≥25 kg/m², *n* = 84)**	***p* value**
	Demographics		
Age [years]	31.3 ± 0.4	31.5 ± 0.4	0.656
BMI [kg/m²] at the beginning of pregnancy	20.3 ± 0.1	30.3 ± 0.4	4.14×10^{-58}
Previous pregnancy (%)	53	57	0.682
Gestational weight gain [kg] (1st to 3rd trimester)	11.19 ± 0.32	11.19 ± 0.46	0.806
	Birth characteristics		
Gestational age [weeks]	39.0 ± 0.1	39.4 ± 0.1	0.044
Weight [grams]	3413.0 ± 51.2	3580.6 ± 46.4	0.017
Height [centimeters]	51.9 ± 0.3	52.2 ± 0.3	0.376
Head circumference [centimeters]	34.9 ± 0.2	35.2 ± 0.2	0.121
Female child in all participants, No. (%)	46 (53.5)	45 (53.6)	0.998
Female child from received cord blood samples, No. (%)	12 (54.6)	8 (47.1)	0.898

2. Circulating parameters

Circulating insulin, leptin, TREM2 and GDF15 levels were measured with a commercial ELISA kit (R&D) according to the manufacturer´s instructions. Cholesterol and high-density lipoprotein (HDL) cholesterol were measured using a commercial kit (DiaSys).

3. Lipidomics

3.1 Lipidyzer

Analysis of structural and storage lipids was performed using the Lipidyzer™ platform from SCIEX. Blood samples were spiked with Lipidyzer™ Internal Standards (SCIEX). Afterwards, lipids were extracted with methyl *tert*-butyl ether/methanol (Matyash et al., 2008). Next, lipids were concentrated with help of a vacuum centrifuge and reconstituted in dichloromethane/methanol (50/50) buffer containing 10 mM ammonium acetate. Lipid extracts were directly infused into an electrospray ionization-triple quadrupole (ESI-QqQ) (QTRAP® 5500; SCIEX) system equipped with a SelexION device to separate lipids by differential mobility spectroscopy and lipids were analyzed in multiple reaction monitoring (MRM) mode. The lipidomics platform was operated using Analyst version 1.6.8 and Lipidomics workflow manager (SCIEX). Data analysis was performed using the Shotgun Lipidomics Assistant software (Su et al., 2021) after converting the WIFF files into an mzML format employing the MSconvertGUI application (Proteowizard) (Chambers et al., 2012).

4.2 Bile acid measurement

1 ml of methanol containing BHT (1 g/l) and 5 µl of internal standard were added to 50 µl of samples. The reaction tubes were vortexed for 15 seconds to homogenize the samples. Second, samples were centrifuged for 10 min at 10.000 g and 4 °C. The supernatant was filtered using a polytetrafluoroethylene (PTFE) filter and transferred into a new 1.5 ml Eppendorf tube. Next, the solvent was evaporated using a vacuum centrifuge. The precipitate was dissolved in 50 µl Eluent B. Finally, samples were transferred into a glass vial and closed. Until measurement, bile acid extracts were stored at -20 °C.

Bile acid quantification was performed by high-performance liquid chromatography (HPLC) coupled to electrospray ionization tandem mass spectrometry (Wegner et al., 2017). For quantification, multiple reaction monitoring (MRM) mode was applied for measurement. NEXERA X2 LC-30AD HPLC PUMP (Shimadzu, Tokyo, Japan) equipped with a Kinetex C18 column (100 Å, 150 mm × 2.1 mm i.d., Phenomenex, Torrance, CA, USA) was used for HPLC analysis. The mobile phase A was HPLC-grade water enriched with 0.1% formic acid and 20 mM ammonium acetate. Mobile phase B consisted of acetonitrile methanol (3/1 v/v) enriched with 0.1% formic acid and 20 mM ammonium acetate. Retention time and specific MRM transitions were compared to the corresponding standard chromatograms in order to identify and quantify individual peaks. Hydrophobicity indices were calculated after Heuman (1989).

4. Statistical analysis

GraphPad Prism software (version 10.0.2, GraphPad Software, Inc., La Jolla, CA, USA) and R (version 4.1.2) were used for statistical analysis. Chi-squared tests were used to determine differences between two groups for categorical variables and Student´s *t* test for unpaired, non-categorical variables of two groups. For the comparison of paired data of longitudinal datasets (i.e. first trimester *versus* second or third trimester) paired *t* tests with Bonferroni correction was performed. Effect size Cohen´s *d* was calculated as difference between mean of both groups divided by the pooled standard deviation. $p < 0.05$ was considered significant and $p < 0.1$ was considered a statistical trend.

Part D: Results

1. Cohort description

As described in the methods section above, blood samples of a prospective pregnancy cohort from each trimester and cord blood (CB) taken at birth were analyzed. Participants were divided according to their first trimester BMI into a “normal BMI” group (BMI <25 kg/m²) or categorized as overweight (BMI ≥25 kg/m²) and matched for age, parity, gestational weight gain and fetal sex.

2. Analysis of circulating hormones

As described in the introduction, leptin, insulin, GDF15 and TREM2 are involved in the regulation of energy metabolism in several tissues. To get an insight into these processes during gestation with regard to overweight, their abundancies within the circulation were measured in samples from pregnant women and the respective cord blood samples using Enzyme-linked Immunosorbent Assay (ELISA).

2.1 Leptin

Leptin was reported to be proportional to body fat mass in non-pregnant individuals since adipose tissue is the major source of circulating leptin (Flier, 1997). It is an important regulator of energy metabolism as it controls appetite and regulates energy expenditure (Campfield et al., 1995). Besides signaling through the central nervous system, leptin signaling was implicated in the regulation of placental lipid metabolism and reproductive complications (Kelesidis et al., 2010; Mousiolis et al., 2012). Thus, analysis of leptin levels in this cohort are considered to replicate previous findings and to verify our study design.

Analysis of circulating leptin revealed significant differences in women with a normal BMI and overweight. In early gestation, median leptin concentration in the circulation from lean women was 25 ng/ml and thus approximately 4-fold lower compared to leptin concentrations in samples from women with overweight. At each trimester, women with overweight displayed higher levels of circulating leptin.

Whereas the concentration of leptin in lean women doubled throughout pregnancy from 25 ng/ml to 50 ng/ml, leptin concentration did not further increase in women with overweight during the course of pregnancy compared to the first trimester. In the third trimester, circulating leptin concentration in women with overweight was twice as high compared to lean control women. However, no difference was not observed in cord blood (CB) between the two groups. Notably, the CB leptin concentration was in a similar range as in the samples from lean women obtained in early pregnancy (Figure 6A). Conclusively, leptin concentration verified our study design with regard to increased leptin levels and resistance in overweight.

2.2 Insulin

Physiological, healthy pregnancy is characterized by peripheral insulin resistance to promote nutrient utilization by the fetoplacental unit in late gestation (Sonagra et al., 2014). Insulin resistance was described in maternal obesity and linked to various adverse obstetric outcomes (Catalano & Ehrenberg, 2006). As a proof of concept, circulating insulin was analyzed in this cohort to detect potential disturbances of transplacental nutrient transport.

In early gestation, median insulin concentration was approximately 120 pmol/ml. Whereas insulin concentration in the circulation of lean control individuals seemed to be stable until the second trimester, it significantly increased in the circulation of women with overweight. In line with that, insulin concentration in the second trimester was significantly higher in the circulation from women with overweight compared to lean participants. However, this difference was not significant in the third trimester, whereas a statistical trend was observed ($p = 0.089$) (Figure 6B). However, the concentration of insulin correlated significantly with maternal BMI at each trimester (Table 2). Notably, third trimester circulating insulin concentration significantly increased compared to insulin levels in early gestation in both groups, which increased approx. 2.7-fold in both groups. As observed for leptin concentrations, insulin concentration was similar in the CB taken from women with and without overweight (Figure 6B). In line with previous reports, these data indicate that women with overweight in our cohort display a slight insulin resistance.

2.3 Growth/differentiation factor 15

Growth/differentiation factor 15 (GDF15) was implicated in the regulation of whole-body energy homeostasis as it signals through GFRAL in the brain to regulate food intake (Yang et al., 2017). Importantly, its expression is linked to the inflammatory status as it is secreted by activated macrophages (Bootcov et al., 1997). GDF15 is also expressed in the placenta (Lawton et al., 1997) and increased during the course of pregnancy (Marjono et al., 2003). Thus, gestational GDF15 might serve as a link between regulation of energy homeostasis and immune status.

In the first trimester, the median concentration of GDF15 in lean women was 6400 and approx. 5400 pg/ml in women with overweight. This difference was less pronounced in the second trimester and completely disappeared in the last trimester. In the third trimester, circulating GDF15 was similar between the two groups (around 12,000 pg/ml) (Figure 6C). This was underscored by an inverse correlation between maternal BMI in the first trimester and GDF15, which was not observed in second (statistical trend) and third trimester (Table 2). Circulating GDF15 in CB seemed to be higher in lean individuals, although this difference was not statistically significant (Figure 6C). These data indicate that GDF15 might have beneficial effects particularly in early pregnancy.

2.4 Triggering receptor expressed on myeloid cells 2 (TREM2)

Triggering receptor expressed on myeloid cells 2 (TREM2) is a phagocytic receptor on macrophages with the capacity to bind lipids (Cannon et al., 2012; Takahashi et al., 2005; Wang, Cella, et al., 2015). Moreover, in non-pregnant individuals AT TREM2 expression correlated with BMI (Reich et al., 2023). However, studies investigating TREM2 abundance in maternal overweight are lacking and potentially give insights into immunometabolic disturbances. Therefore, circulating levels of soluble TREM2 were determined.

The circulating concentration of TREM2 was similar in women with a normal BMI and overweight in the first two trimesters. However, compared to the first trimester, TREM2 increased in both groups during gestation. Whereas the concentration in early gestation was around 1700 pg/ml, it increased to 2120 pg/ml until late pregnancy in the circulation of lean women. In maternal overweight, its

concentration increased from 1500 pg/ml to 2640 pg/ml during the course of pregnancy. This more pronounced increase in maternal overweight is in line with the statistically significant difference in the third trimester (Figure 6B). Similarly, maternal BMI correlated with circulating levels of TREM2 only in the third trimester, but not in the first two trimesters (Table 2). The increasing levels during pregnancy and higher levels in individuals with overweight suggest a role of TREM2 in late pregnancy lipid handling and tissue remodeling.

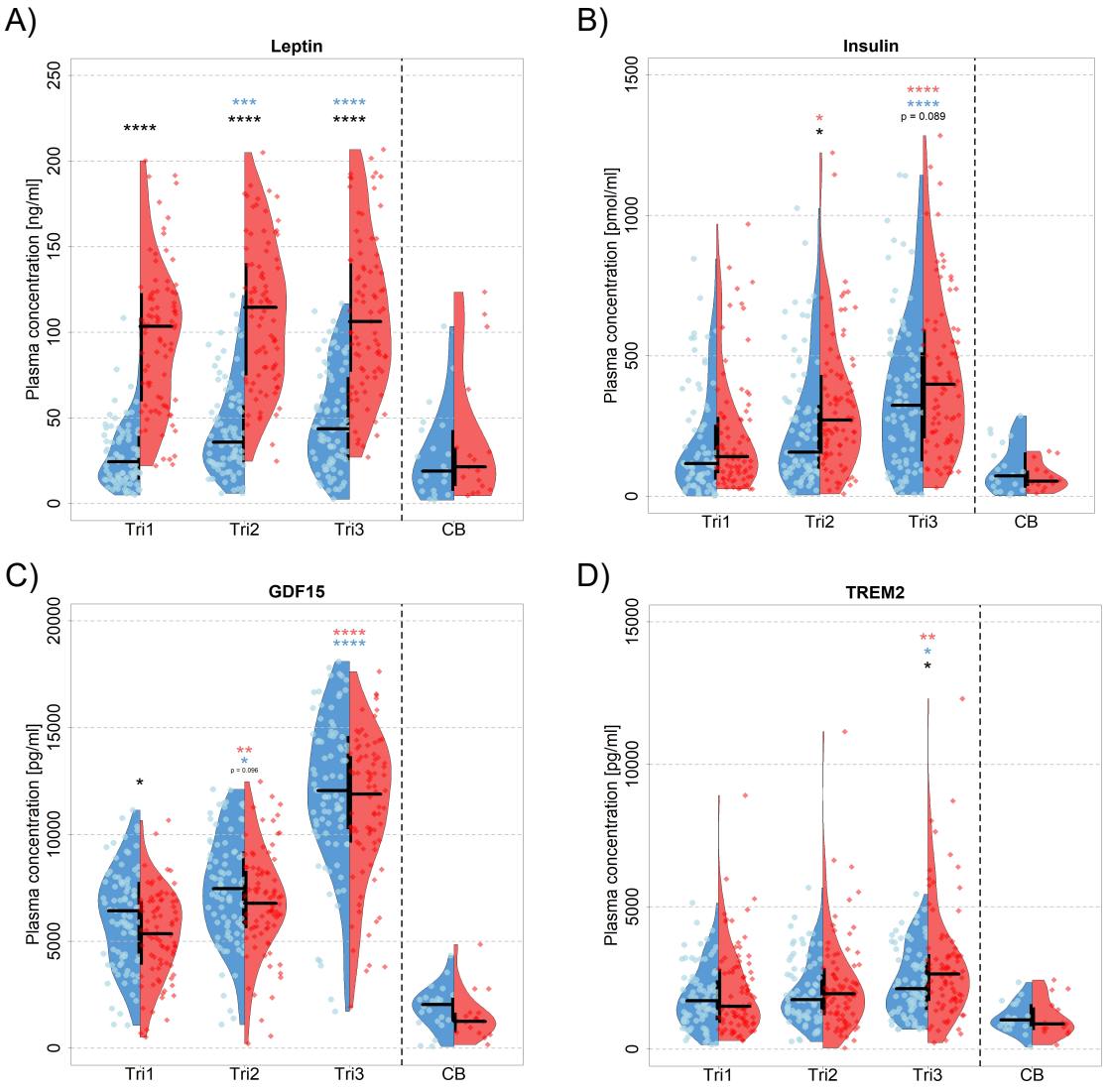

Figure 6: Plasma parameters during pregnancy.
Concentration of A) leptin, B) insulin, C) GDF15 and D) TREM2 at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of respective group. Horizontal black lines represent the median and vertical black lines represent the interquartile range for each group. Black asterisks and *p* values represent difference between normal BMI and overweight at the respective timepoint as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test with Bonferroni correction. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

Table 2: Correlation of circulating hormones with maternal BMI.

Pearson correlation coefficient *r* was calculated between circulating concentration of each hormone and the BMI in the respective trimester. CI: confidence interval; **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; Tri1, 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester.

		Tri1	Tri2	Tri3
Leptin	***r***	0.762	0.751	0.704
	95% CI	0.690 to 0.819	0.675 to 0.812	0.616 to 0.775
	***P* (two-tailed)**	<0.0001 ****	<0.0001 ****	<0.0001 ****
Insulin	***r***	0.204	0.253	0.249
	95% CI	0.053 to 0.346	0.102 to 0.393	0.098 to 0.389
	***P* (two-tailed)**	0.0084 **	0.0013 **	0.0015 **
GDF15	***r***	-0.241	-0.143	-0.123
	95% CI	-0.379 to -0.092	-0.291 to 0.013	-0.273 to 0.033
	***P* (two-tailed)**	0.0018 **	0.0723	0.1222
TREM2	***r***	0.068	0.089	0.206
	95% CI	-0.089 to 0.222	-0.071 to 0.243	0.051 to 0.351
	***P* (two-tailed)**	0.396	0.2748	0.0098 **

3. General lipidomic characterization

To determine the circulating lipid pool, we made use of the Lipidyzer™ platform. This lipidomic approach enables the discrimination of 1,000 structural lipids in 13 classes. In the following chapter, the generated lipidomic data will be used to characterize the study cohort. Afterwards, differences between samples taken from women with a normal BMI or overweight will be evaluated with help of the concentration of each lipid class. Additionally, the percentage of each lipid class of the total lipid pool will be used to describe difference in the lipid pool composition. A more detailed analysis of the individual lipid species and the percentage within the respective lipid class follows this general description.

3.1 The lipid profile differs between lean and overweight mothers

The total lipid pool was lower in the circulation of lean participants compared to overweight individuals but were similar in the second and third trimester. Notably, in both groups the circulating lipid pool increased in the second and third trimester compared to early gestation. However, lipid pool size in CB was not associated with

maternal overweight (Figure 7A). Lipids could be of dietary origin or derived during DNL, which might contribute to the increasing gestational lipid pool. Thus, the lipogenic index was calculated as the ratio between mainly DNL-derived palmitoleic acid (C16:1) and the essential FA linoleic acid (LA, C18:2) as described previously (Worthmann et al., 2024). The lipogenic index was associated with maternal overweight in the first two trimesters, but not in late pregnancy nor in CB. Additionally, the lipogenic index increased in both BMI groups during the course of pregnancy. Notably, while median maternal lipogenic indices rose to max. 0.15, CB lipogenic indices seemed to be higher at approx. at 0.4 (Figure 7B). The difference in lipogenic index was mainly attributed to increased levels of palmitoleic acid rather than altered dietary intake of LA (compare Part D, Results, chapter 5.3, Figure 19A for LA (below) and appendix Figure 22 for palmitoleic acid).

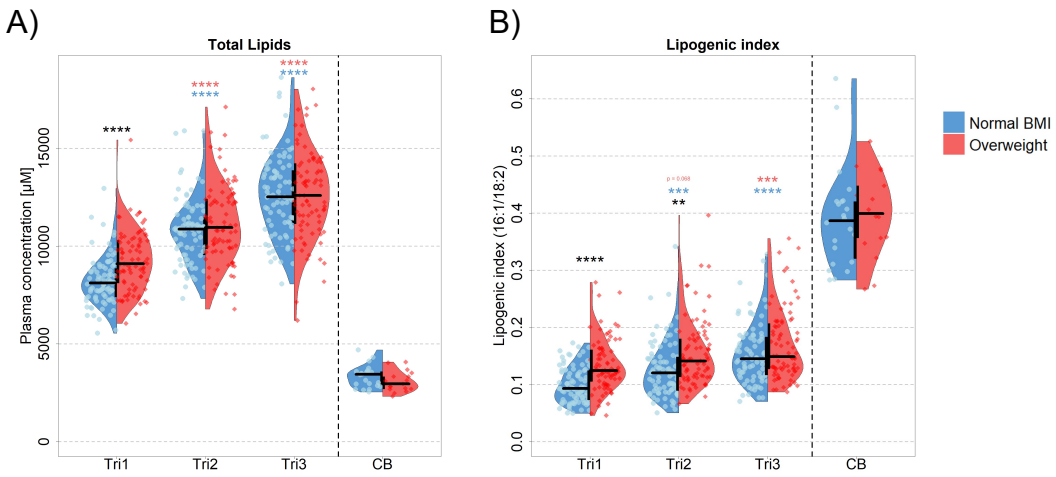

Figure 7: Circulating lipid pool and lipogenic index during pregnancy.
Concentration of A) total lipids and B) lipogenic index at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of respective group. Horizontal black lines represent the median and vertical black lines represent the interquartile range for each group. Black asterisks and *p* values represent difference between normal BMI and overweight at the respective timepoint as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test with Bonferroni correction. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001

Next, principal component analysis (PCA) using the concentration of individual lipid species was performed to reduce dimensionality of the large lipidomic dataset in an unbiased approach. PCA revealed a gradual discrimination between the timepoints by principal component one. CB samples clustered apart from maternal samples. As pregnancy progressed, dispersion of samples increased (Figure 8A). No clear separation between samples from lean participants and women with overweight could be achieved by PCA of all lipid species, although the location of centroids seemed to differ between samples from individuals with a normal BMI and overweight (Figure 8B). However, quantification of the distribution along principal component one and two revealed significant differences between the two groups (p = 0.003 for principal component one and p = 0.011 for component two). The respective density estimations are shown in the appendix (see appendix, Figure 23A, B). Notably, TAG species were the major determinants for the longitudinal separation of maternal samples in principal component one. In contrast, some FFA, DAG and phospholipid (PL) species mainly contributed to the separation of CB samples from maternal samples (Figure 8C). Notably, PUFA-containing FFA and lyso-PLs were the most important variables for component one (data not shown).

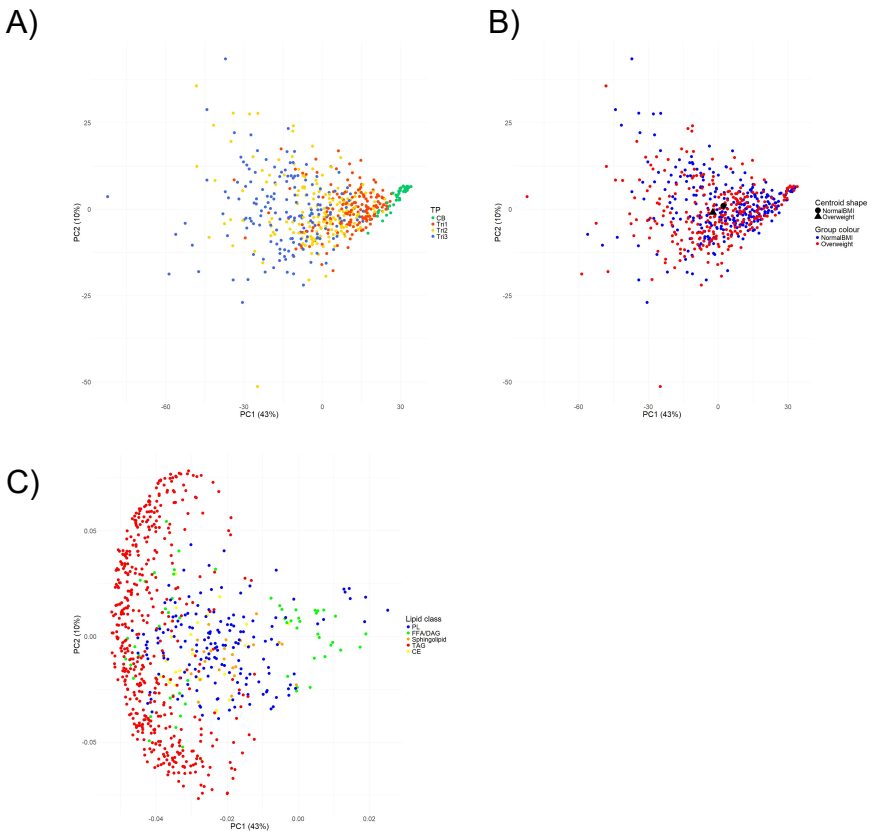

Figure 8: Principal component analysis using lipid species concentration.
Illustration by A) timepoints (TP) and B) maternal BMI group. Centroid of normal BMI group is indicated by a black circle and of overweight group by a black triangle. C) Loading plot of PCA. Lipid classes are highlighted in different colors. Explained variance of each principal component is indicated in brackets.

Similarly, we used the contribution of individual lipid species to their respective lipid classes for PCA. PCA revealed a more pronounced separation between maternal and CB samples across principal component one. Moreover, a gradual progression for the longitudinal discrimination of maternal samples was observed across principal component two (Figure 9A), whereas no clear separation of maternal BMI groups was achieved. However, quantification of the distribution of the two groups across the two components revealed that principal component two ($p = 0.006$), but not principal component one ($p = 0.252$), has the potential two explain the variance between the two BMI groups (Figure 9B). The respective density estimations are shown in the appendix (see appendix, Figure 23C, D). The separation of CB from maternal samples might be related to some FFA and DAG species, whereas the separation of maternal samples across principal component two might be partially attributed to distinct TAG species (Figure 9C).

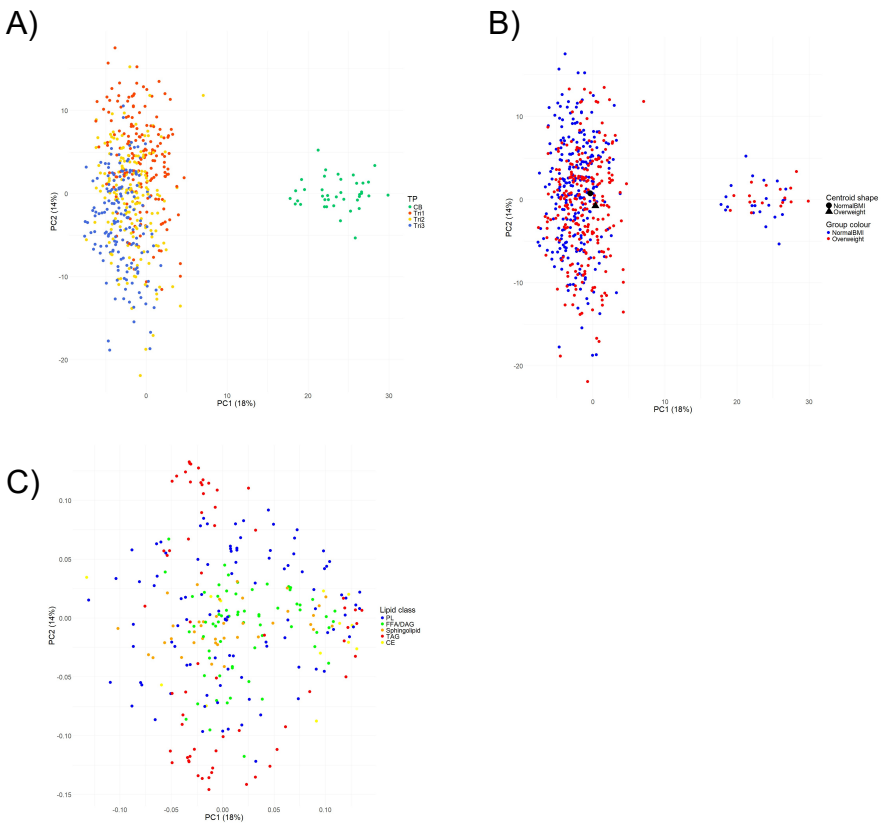

Figure 9: Principal component analysis using lipid species composition.
Illustration by A) timepoints (TP) and B) maternal BMI group. Centroid of normal BMI group is indicated by a black circle and of overweight group by a black triangle. C) Loading plot of PCA. Lipid classes are highlighted in different colors. Explained variance of each principal component is indicated in brackets.

In summary, gestational timepoint seemed to have a stronger impact on the lipidome than maternal BMI and the concentration of TAG species are major determinants for the longitudinal discrimination of maternal samples, whereas some PLs, FFAs and DAGs might further discriminate maternal from CB samples. Notably, the composition of the individual lipid classes separated CB from maternal samples even more pronounced, and PUFA-lipids (i.e. incorporated into FFA, lyso-PLs) seemed to be the major determinants in that context. For the discrimination between lean individuals and women with overweight, more detailed analysis will be performed in chapter 4 and 5 (below). The following chapters will focus on the abundancy of different lipid classes during pregnancy.

3.2 Highly abundant neutral lipids are altered in maternal overweight

The concentration of CE in the circulation of lean women was higher in the first trimester compared to women with overweight. However, CE concentration increased in both groups until the second and third trimester compared to early gestation. In mid-pregnancy, the concentration of CE was similar between the two groups, whereas maternal overweight was associated with lower CE concentration in the third trimester. The concentration of CE in the CB was unaffected by maternal BMI (Figure 10A). In the first trimester, CE makes up 40% of the lipid pool in lean women and this percentage is higher compared to women with overweight. This difference was also persistent in the second and third trimester, but not in CB. Of note, the contribution of CE to the total lipid pool decreased in both groups compared to the first to third trimester (Figure 10B).

The concentration (Figure 10C) and composition of TAG (Figure 10D) were associated with maternal overweight at each trimester and increased in both groups during gestation. The concentration of TAG almost doubled during pregnancy in both lean women and participants with overweight (Figure 10C). Notably, the cumulative proportion of CE and TAG of the total lipid pool was higher than 50% at each trimester (compare Figure 10B and D). However, neither the concentration (Figure 10C) nor the composition (Figure 10D) of TAG differed in CB between the two groups.

For both the concentration (Figure 10E) and the proportion of DAG (Figure 10F) there was no difference in samples taken from women with a normal BMI or overweight at any evaluated timepoint. However, the circulating concentration of DAG was approx. doubled in the third trimester compared to early gestation in both groups (Figure 10E). Of note, the proportion of the total lipid pool of DAG remained lower than 1% for almost all samples that were analyzed (Figure 10F). The circulating concentration of FFA was similar between lean women and participants with overweight at each timepoint and the median concentration fluctuated around 400 µM. Notably, the concentration of circulating FFA in CB seemed to be in a similar range compared to maternal samples (Figure 10G). Regarding the contribution of FFA to the total lipid pool, no association with maternal BMI was observed. However,

the proportion significantly decreased in both groups during gestation from initially 5% to 3.5%. Notably, the proportion of FFA to the CB lipidome seemed to be higher at approx. 12% compared to the maternal lipidome (due to the distinct nature of the CB potential differences were not tested between maternal and CB samples) (Figure 10H). Altogether, this demonstrates that maternal overweight is associated with higher levels of TAG. Moreover, half of the maternal lipidome consists of TAG and CE, but especially in late pregnancy lean individuals have more CE in their circulation. Additionally, FFAs contribute to a higher percentage to the CB lipidome.

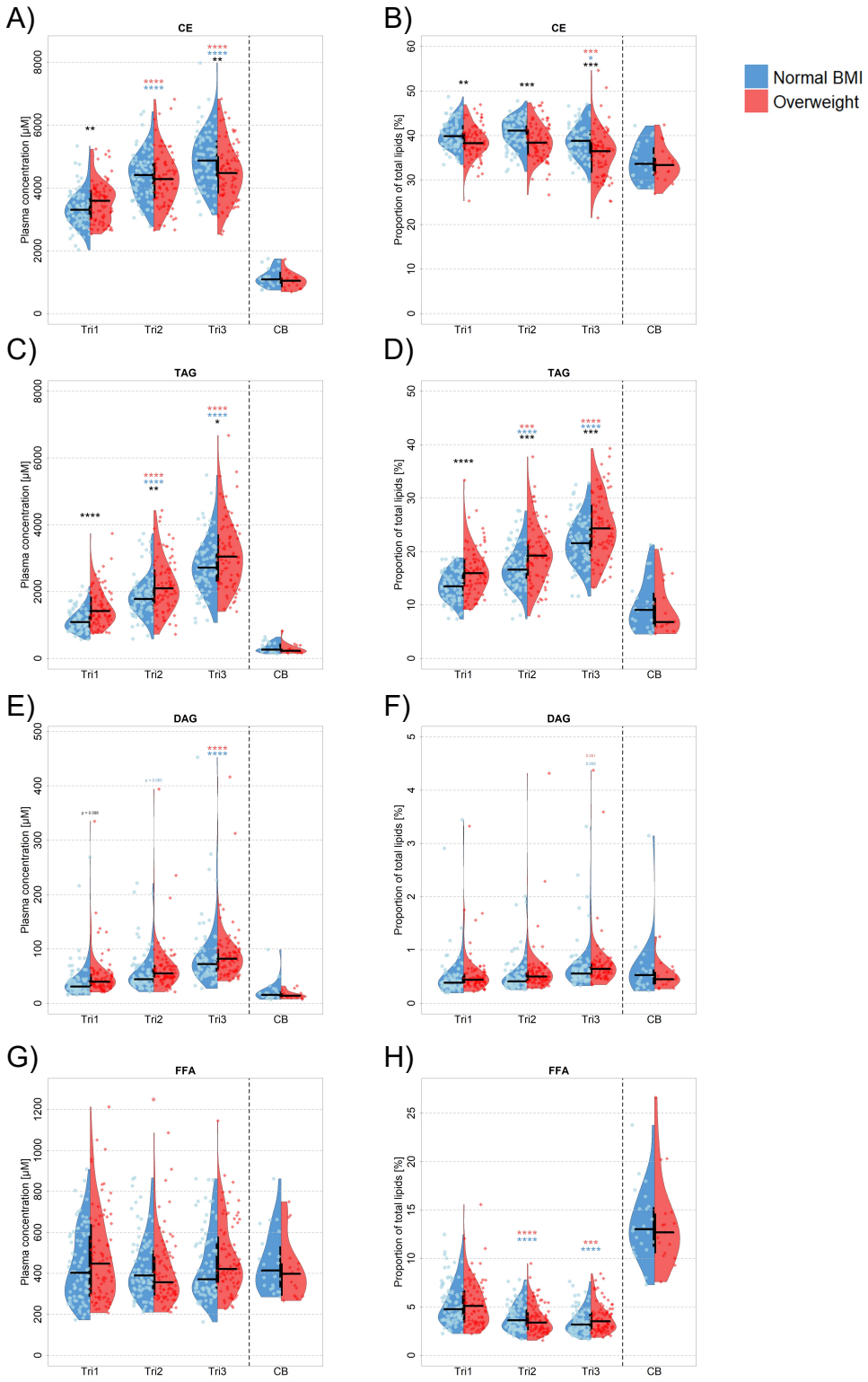

Figure 10: Neutral lipids during pregnancy and in cord blood.
Concentration of A) CE, C) TAG, E) DAG and G) FFA as well as their respective proportions of the total lipid pool B), D), F), H) at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of groups. Black asterisks and *p* values represent difference between normal BMI and overweight as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

3.3 The glycerophospholipid profile differs between mothers and CB

Glycerophospholipids (PLs) are emulsifiers for hydrophobic substances (J. Li et al., 2015) and implicated in the transplacental transport of nutrients (Powell et al., 2024). Thus, analysis of PL within the circulation provides insights into the transplacental transport of maternal lipids.

In the first trimester, PC concentration was higher in women with overweight compared to lean individuals, whereas this difference was neither observed in second or third trimester nor in CB. Notably, the concentration of PC increased in both groups during gestation from approx. 2,500 µM in lean participants and 2,700 µM in women with overweight to approx. 3,200 µM in the third trimester in both groups. The concentration of PC in CB was around 1,000 µM (Figure 11A). However, an inverse pattern was observed for the proportion of PC of the total lipid pool. Hence, first trimester abundance was higher in lean women compared to counterparts with overweight. Moreover, the proportion decreased during pregnancy in both groups from approx. 30% to 26%. 28% of the CB lipidome consisted of PC (Figure 11B). The concentration of hydrolyzed PC, LPC, was not associated with maternal BMI. However, there was a significant decrease in gestational LPC, which was observed for both groups. CB LPC seemed to be in a similar range as observed in the maternal circulation at first trimester around 160 µM (Figure 11C). The contribution of LPC to the total lipid pool showed a similar pattern as observed for its putative precursor PC, including a negative association with maternal overweight in the first trimester and gestational decrease in both groups. Of note, less than 2% of the first trimester lipidome consisted of LPC, whereas 5% of the CB lipids are LPC species (Figure 11D).

The concentration of PE was associated with maternal overweight at each trimester, but not in CB taken at birth. Moreover, it increased in both groups during gestation (Figure 11E). Similarly, the contribution of PE to the total lipid pool was higher in women with overweight in mid and late pregnancy, but not in the first trimester or CB. Moreover, its proportion of the lipid pool increased during gestation from 2.1% to 2.5% in lean individuals and from 2.2% to 2.7 in participants with overweight. In CB taken at birth, 1.3% of the lipid species belong to the class of PE

(Figure 11F). Notably, the concentration of the putative PE-derivate LPE seemed to be unaffected by maternal BMI or the timepoint the sample was derived as it fluctuated around 4.1 µM in all groups both in gestation and at birth (Figure 11G). Similarly, its contribution to the lipid pool was not associated with maternal BMI at any timepoint analyzed. However, there was a significant decrease during gestation in both groups. Whereas approx. 0.05% of first trimester lipids were LPE species, they made up 0.12% of CB lipids (Figure 11H). In conclusion, PC and PE increase during gestation and especially PE is associated with maternal overweight. However, their hydrolyzed derivates LPC and LPE seemed to contribute more to the CB lipidome than to the maternal one.

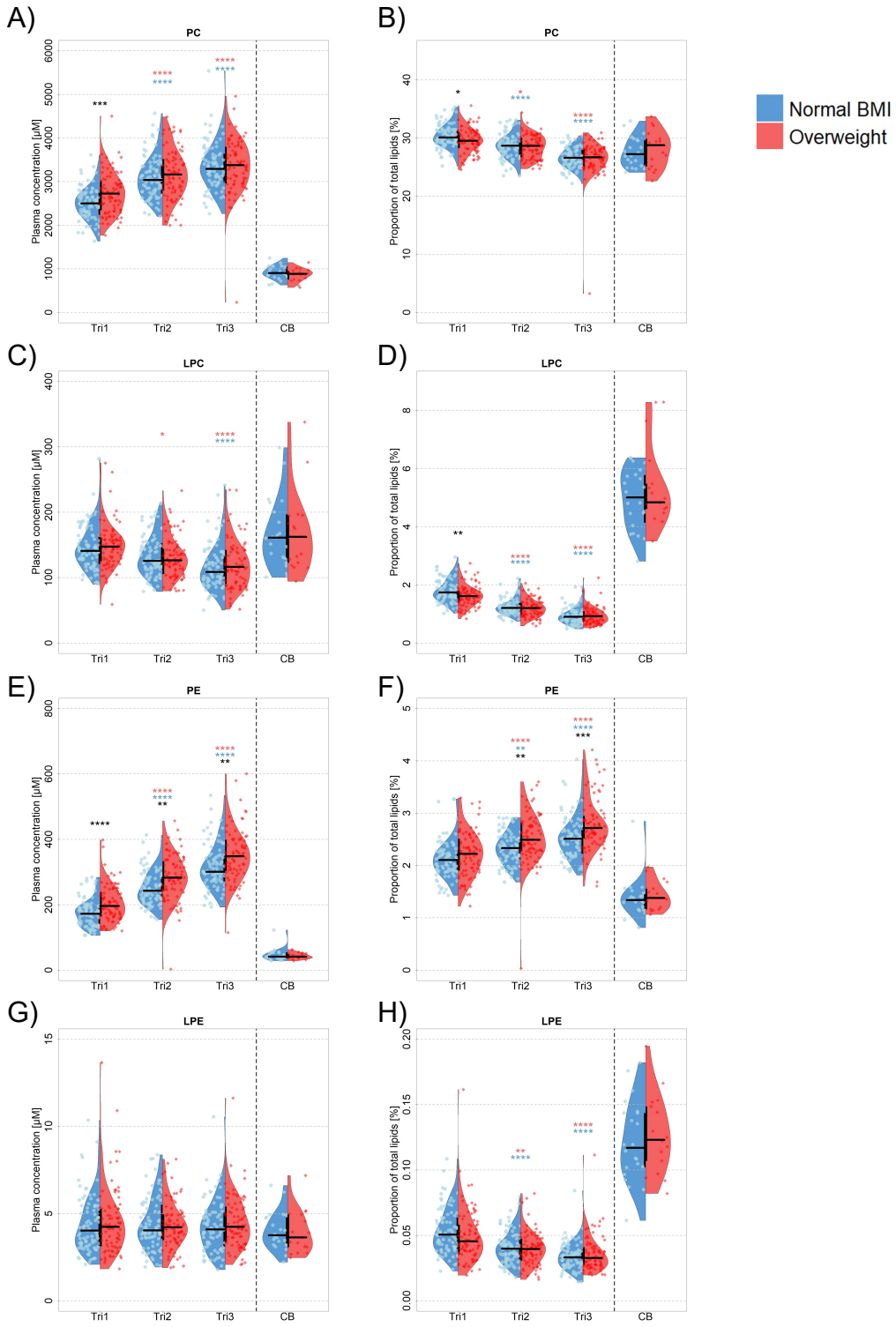

Figure 11: Glycerophospholipids during pregnancy and in cord blood.
Concentration of A) PC, C) LPC, E) PE and G) LPE as well as their respective proportions of the total lipid pool B), D), F), H) at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of groups. Black asterisks and *p* values represent difference between normal BMI and overweight as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

3.4 The sphingolipidome during pregnancy

Sphingolipids are important second messengers and implicated in physiological pregnancy (Enthoven et al., 2023), but disbalance of sphingolipid metabolism was demonstrated in preeclampsia and maternal overweight (Dobierzewska et al., 2017; León-Aguilar et al., 2019). Additionally, offspring from women with overweight seem to inherit this dysregulated sphingolipid metabolism (León-Aguilar et al., 2019). Consequently, analysis of the maternal sphingolipidome might reflect offspring long-term sphingolipid metabolism in our cohort as well.

In early gestation, the concentration of CER tended to be higher in women with overweight compared to their lean counterparts. However, this association with BMI was absent in samples taken in mid to late pregnancy and CB. However, the concentration of CER rose in both groups during gestation (Figure 12A). Its proportion of the lipidome was similar in all conditions we analyzed and varied around 0.05% (Figure 12B). Similarly, the concentration of DCER was not associated with maternal overweight and increased from first to third trimester in both groups (Figure 12C). Its gestational proportion of the lipidome was around 0.01% and did not differ between lean individuals and their counterparts with overweight. The proportion of DCER of the CB lipidome was higher than 0.02% in both groups. Moreover, it tended to be higher in CB samples from mothers with overweight, although there was a large variation in CB from women with overweight (Figure 12D). The concentration of the glycosphingolipid HCER increased during gestation independently of maternal BMI. However, in late pregnancy it tended to be higher in the circulation of lean participants, whereas there was no difference at any other timepoint that was evaluated (Figure 12E). The composition of HCER of the total lipid pool was higher in lean women compared to participants with overweight in the first trimester. Since there was neither an increase nor a decrease during gestation, the proportion of HCER of the lipidome varied around 0.05%, including both gestational and CB samples (Figure 12F). The abundance of LCER in the gestational circulation was around 5 µM and in a similar range as observed for the other glycosphingolipid HCER (compare Figure 12E and G). Moreover, LCER concentration was not associated with maternal BMI or gestational timepoint (Figure 12G). As observed for

HCER, the proportion of lipids that are LCER was higher in lean individuals in early pregnancy and decreased independently of BMI during gestation (Figure 12H). An increased concentration of SM was associated with maternal overweight in the first trimester, but not in mid or late pregnancy. SM increased during pregnancy and reached a concentration of 600 µM in both groups in the third trimester. However, its concentration in CB was around 250 µM (Figure 12I). During the course of pregnancy, the proportion of lipid species that belong to the group of SM decreased from approx. 5.6% to 4.9% and was not associated with maternal BMI. Notably, approx. 8% of the CB lipidome consists of SM (Figure 12J).

Conclusively, most sphingolipids increased during pregnancy, but their contribution to the maternal lipidome remained below 1% for most of the sphingolipids. However, the most abundant sphingolipid SM was higher in maternal overweight in early gestation. Conversely, complex glycosphingolipids contributed to a greater extend to the early gestational lipidome in lean individuals.

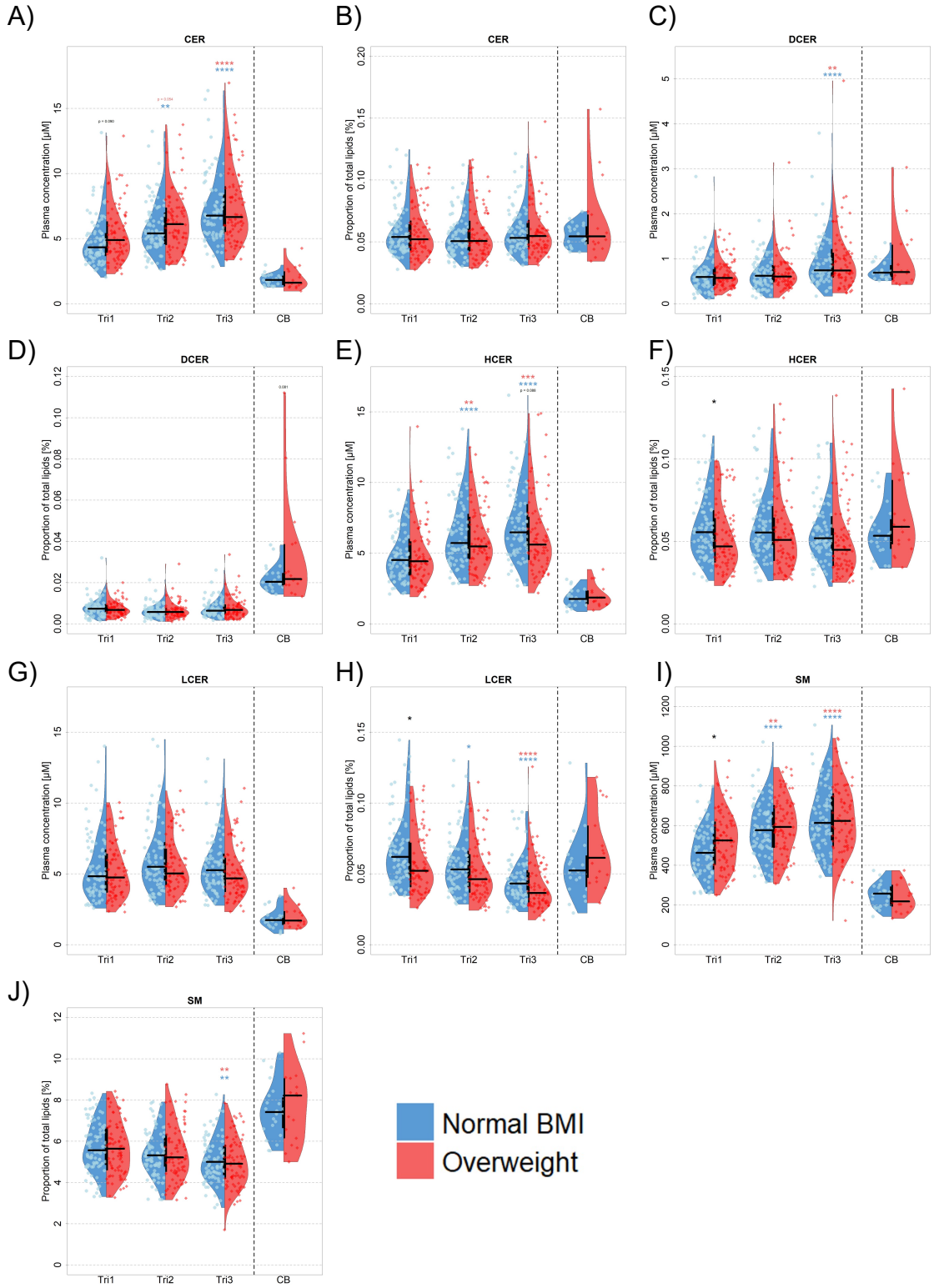

Figure 12: Sphingolipids during pregnancy and in cord blood.
A) concentration of CER, B) its proportion of the total lipid pool, C) concentration of DCER, D) its proportion of the total lipid pool, E) concentration of HCER, F) its proportion of the total lipid pool, G) concentration of LCER, H) its proportion of the total lipid pool, I) concentration of SM and J) its proportion of the total lipid pool at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of groups. Black asterisks and *p* values represent difference between normal BMI and overweight as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

3.5 GDF15 and TREM2 correlate with circulating lipids

As oral gavage of FAs increases GDF15 expression and GDF15 in turn induces lipolysis in AT (Chrysovergis et al., 2014; Kanta et al., 2023; Laurens et al., 2020; Wang et al., 2024), we hypothesized that gestational GDF15 might be related to circulating FFA. At each trimester, GDF15 moderately correlated with FFA. However, this was not observed in CB. Additionally, maternal GDF15 correlated positively with sphingolipids, i.e. CER, LCER and SM. Notably, high-density lipoprotein was inversely associated with GDF15 in first and third trimester (Figure 13A).

TREM2 was shown to bind lipids directly, which includes among others PC, PE, HDL, and SM (Cannon et al., 2012; Song et al., 2017; Wang, Cella, et al., 2015). Moreover, soluble TREM2 in patients with coronary heart disease was recently associated with circulating TAG, total cholesterol, and HDL-cholesterol (Liu et al., 2023). Thus, we hypothesized that gestational TREM2 in the circulation is associated with these parameters. Unexpectedly, soluble TREM2 neither correlated with PC or SM nor with related lipid classes (i.e. hydrolyzed LPC or ceramides) during pregnancy. However, concentrations of TREM2 and PE positively correlated in mid-pregnancy and in CB samples. Instead, TREM2 was strongly associated with DAG, TAG, and cholesterol during pregnancy. Of note, TREM2 and HDL were inversely correlated during pregnancy, whereas in CB they had a positive association (Figure 13B).

Conclusively, GDF15 was associated with FFAs, suggesting either its induction by FFA and/or a role in lipolytic processes. In contrast, TREM2 seems to be rather unrelated from sphingolipids, whereas it might be implicated in the processing of lipoprotein particles and related lipids (i.e. cholesterol, TAG).

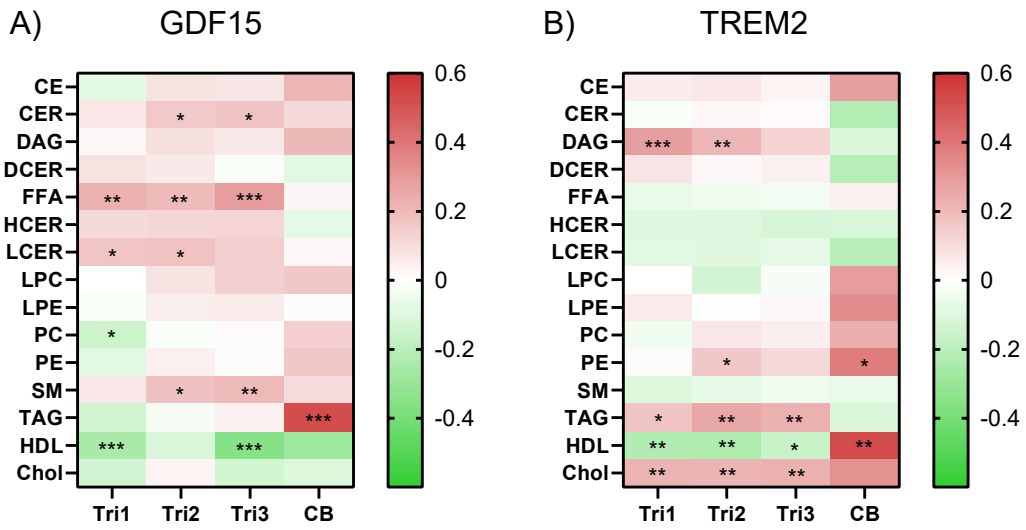

Figure 13: Correlation of GDF15 and TREM2 with plasma lipids.
Pearson correlation coefficient *r* was calculated between circulating concentration of A) GDF15 and B) TREM2 and lipids at the respective trimester. Correlation coefficient is represented by a two-color coded scale. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; Tri1, 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood.

3.6 Bile acids

BAs have cytotoxic potential, which is partially determined by their conjugation state (Thomas et al., 2008). Moreover, gestational BA accumulation in pathological situations such as ICP was linked to an increased risk of GDM and preterm birth (Liu et al., 2020). Conjugated BAs (CBA) was not associated with maternal BMI in our cohort. Moreover, the concentration remained around 2 µM during gestation and in the CB taken at birth (Figure 14A). Similarly, gestational levels of unconjugated BAs (UBA) were not associated with maternal overweight and remained around 0.3 µM during pregnancy. However, CB from lean mother seemed to possess more UBA compared to CB taken from women with overweight (p = 0.081) (Figure 14B). Hence, the gestational conjugation pattern of BAs seemed to be similar between lean participants and women with overweight. Moreover, the concentration of CBA and UBA seemed to be in a similar range in maternal and CB samples, suggesting similar transplacental transport of BAs.

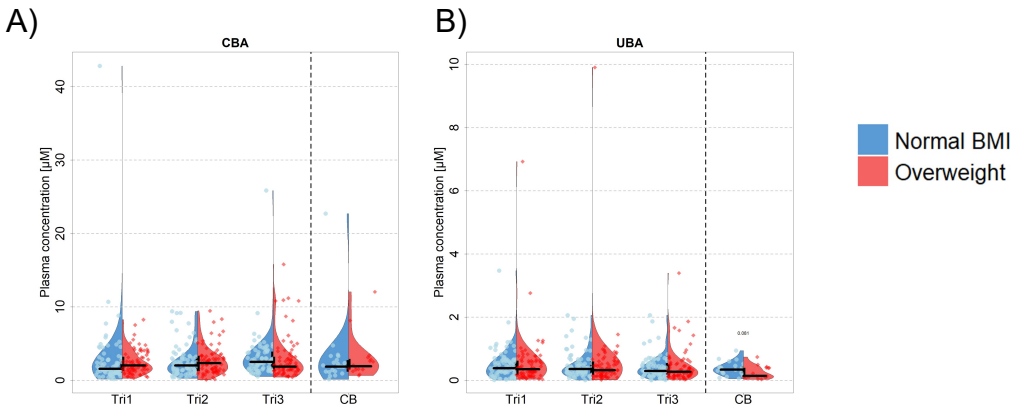

Figure 14: Circulating BA pool during pregnancy.
Concentration of A) conjugated BAs and B) unconjugated BAs at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of respective group. Horizontal black lines represent the median and vertical black lines represent the interquartile range for each group. Black asterisks and p values represent difference between normal BMI and overweight at the respective timepoint as determined by Student´s t test. Blue as asterisks and p values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired t test with Bonferroni correction. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

4. Analysis of individual lipid species during maternal overweight

4.1 Maternal overweight is associated with altered linoleic acid and arachidonic acid lipids

After this general characterization of the gestational and CB lipidome, we wanted to go more into detail. Thus, we screened through the 1,000 lipid species and determined if there are differences between samples from participants with a normal BMI and those with overweight.

In the first trimester, 422 lipids were significantly (p = 0.01) higher in the circulation from women with overweight, whereas one lipid (dilinoleoyl phosphatidylcholine, PC(18:2/18:2)) was higher in lean individuals. Lipids that were higher in maternal overweight included mainly TAG and PE species esterified predominantly to arachidonic acid (AA, C20:4), oleic acid (C18:1) and stearic acid (C18:0) (Figure 15A). To test if these differences were potentially of biological relevance, the effect size Cohen´s d was determined. Analysis of effect size underscored that linoleic acid (LA, C18:2) incorporated into phospholipids was associated with a normal BMI compared to maternal overweight. Notably, the Cohen´s d for PE(18:2/18:3) was greater than 2, which indicates a strong effect. Moreover, other PUFAs such as eicosadienoic acid (C20:2) and linolenic acid (C18:3) incorporated into phospholipids were part of the lipid signature in lean individuals. Additionally, the effect sizes for the lipid species associated with maternal overweight were approx. at Cohen´s d higher than one, indicating a strong effect (Figure 15B).

In the second trimester, 129 lipids were significantly (p = 0.01) higher in maternal overweight, whereas ten were higher in the normal BMI group. As observed for the first trimester, maternal overweight was positively associated with TAG and PE species containing stearic acid and AA, but negatively associated with phospholipids esterified to PUFAs (e.g. LA, linolenic acid, docosahexaenoic acid (DHA)) (Figure 15C). This pattern was also underscored by analysis of the respective effect sizes. Additionally, HCER esterified to oleic acid was higher in lean individuals, whereas its SM analogue was higher in women with overweight (Figure 15D).

In the third trimester, 72 lipids were significantly (p = 0.01) higher in individuals with overweight, whereas 16 lipids were higher in lean women. Again, maternal overweight was characterized by higher AA and stearic species incorporated into TAG and phospholipids. In addition to the observations from the first two trimesters, the lipidome of lean individuals was characterized by higher CE species containing odd-chain FAs pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) as well as even-chain FAs dodecanoic acid (C12:0), tetradecanoic acid (C14:0) and LA. Moreover, CER esterified to the very long-chain FA (VLCFA) hexacosanoic acid (C26:0) was higher in lean individuals compared to women with overweight (Figure 15E). Analysis of effects sizes underscored the observed differences between lean women and participants with overweight. Additionally, maternal overweight was associated with higher levels of several ether phospholipids and DAG species containing a saturated FA (i.e. stearic acid, palmitic acid, tetradecanoic acid) and a PUFA (i.e. linolenic acid, eicosadienoic acid, DHA) with large effect sizes (Cohen´s d greater than 0.8) (Figure 15F).

In CB, no lipid was significantly (p = 0.01) higher in the overweight group, whereas five lipids were higher in the lean group. Among these five lipids, PUFAs from different lipid classes predominated. This included LA-containing PE(16:0/18:2), DAG(18:2/20:3) and TAG. Additionally, CE containing the FA C14:1 and the hydrophilic BA GHCA were identified (Figure 15G). Consistently, medium (Cohen´s d between 0.5 and 0.8) to large effect sizes were observed for several phospholipids and TAGs containing either LA or linolenic acid in lean individuals. However, PUFA-containing lipids such as PC(15:0/18:2), LPC(22:6) or non-esterified stearidonic acid (C18:4) were higher in CB from women with overweight (Figure 15H).

Conclusively, maternal overweight seems to be characterized by a distinct lipidomic profile throughout gestation, which includes higher levels of AA, oleic acid and stearic acid incorporated into TAG and PE species and lower levels of phospholipids enriched in PUFAs such as LA. However, these differences became less pronounced as pregnancy progressed. The CB lipidome was almost similar between the two groups but shared some of the differences described in the maternal circulation (i.e. higher LA and linolenic acid in lean individuals).

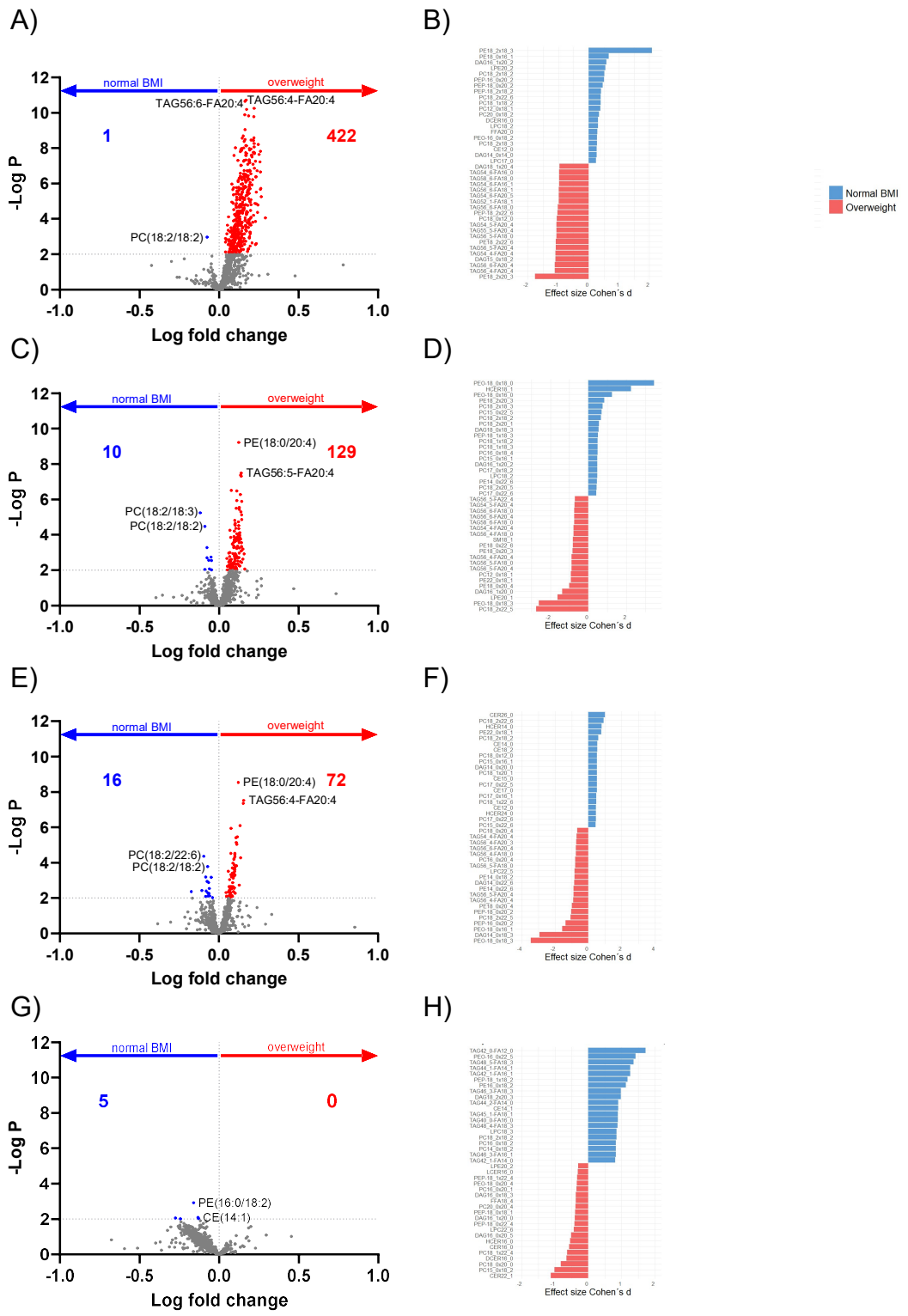

Figure 15: Differences in lipid species concentration during pregnancy and in CB.
Differences in the concentration of lipid species in the circulation of women with a normal BMI and overweight in A) 1st trimester, C) 2nd trimester, E) 3rd trimester and G) CB. Lipids that are significantly higher in lean individuals are highlighted in blue, whereas overweight-associated lipids are highlighted in red ($p < 0.01$ which equals -log$P > 2$). The respective 20 highest effect sizes (Cohen´s d) were calculated for B) 1st trimester, D) 2nd trimester, F) 3rd trimester and H) CB.

4.2 The composition of the sphingolipidome shifts towards shorter acyl chains in maternal overweight

Next, we analyzed the contribution of the individual lipid species to their respective lipid class, thus providing insights into changes of FA profiles within lipid classes. Moreover, this analysis minimizes the effects of overweight-associated hyperlipidemia (as illustrated in Figure 7A), thus enforcing detection of changes in less abundant lipid classes (e.g. sphingolipids).

In the first trimester, the proportion of 75 lipids within their respective lipid class were significantly (p = 0.01) higher in the circulation from women with overweight, whereas the proportion of 119 lipids was higher in lean individuals. Maternal overweight was associated with higher proportions of AA containing lipids, including TAG and CE, and several additional CE species. Moreover, the proportion of CER esterified to stearic acid was higher in women with overweight. In contrast, the circulating phospholipidome of lean individuals consisted to a higher percentage of LA-containing species and ether phospholipids (Figure 16A). These findings were reinforced by analysis of effect sizes, were most of the lipids could be recapitulated. Additionally, the esterification of HCER to the VLCFA tetracosanoic acid (C24:0) was higher in lean individuals, whereas incorporation of tetradecanoic acid (C14:0) was lower (Figure 16B).

In the second trimester, the proportion of 42 lipids within their respective lipid class were significantly (p = 0.01) higher in women with overweight, whereas 147 lipids were higher in lean participants. Among these 42 species associated with maternal overweight, AA containing species dominated. Moreover, the proportions of CER and SM esterified to stearic acid and HCER esterified to palmitic acid were higher in women with overweight compared to lean counterparts. Conversely, the proportions of TAG and PC species containing LA, linolenic acid and oleic acid were higher in lean individuals (Figure 16C). These patterns were also seen when analyzing effect sizes. Additionally, medium to large effect sizes suggest higher levels of the glycosphingolipids LCER(C20:1) and HCER esterified to oleic acid in women with overweight (Figure 16D).

In the third trimester, the proportion of 34 lipids within their respective lipid class were significantly (p = 0.01) higher in individuals with overweight, whereas 105 lipids were higher in lean individuals. As observed for early and mid-pregnancy, maternal overweight was characterized by higher proportions of AA and lower proportions of LA incorporated into TAG and PLs. However, higher proportions of ether PE species containing stearic acid at the ether bond and either DHA or LA at the other *sn*-position were observed in lean participants (Figure 16E). Additionally, a normal BMI was associated with higher proportions of VLCFAs (C26:0, C24:0) incorporated into CER and HCER and, additionally, TAG esterified to tetradecanoic acid compared to women with overweight as indicated by medium to large effect sizes (Figure 16F).

In CB, the proportion of two lipids within their respective lipid class were significantly (p = 0.01) higher in individuals with overweight, whereas 16 lipids were higher in lean individuals. Proportions of the PE ether lipid esterified to docosatetraenoic acid (C22:4) at the *sn*-2 position and the hydrophobic BA tauro-chenodeoxycholic acid (TCDCA) were higher in maternal overweight. Conversely, CB of lean individuals contained higher proportions of several TAG species esterified to unsaturated C18-FAs (linolenic acid, LA, oleic acid) and to saturated FAs pentadecanoic acid and tetradecanoic acid compared to CB samples from women with overweight. Additionally, the proportion of hydrophilic GHCA within BAs and the proportion of CE(14:1) were higher in lean individuals (Figure 16G). However, low to medium effect sizes were observed for higher proportions of ceramide species esterified to palmitic acid or erucic acid (C22:1) (LCER, HCER, CER, DCER) and several plasmalogen species in women with overweight compared to lean counterparts (Figure 16H).

In summary, maternal overweight was characterized by increased proportions of AA, stearic acid and palmitic acid incorporated into TAG and phospholipids as well as lower proportions of linolenic acid-, LA- and oleic acid-containing TAG and phospholipids. Notably, PUFAs incorporated into ether phospholipids were predominantly found in lean individuals, altogether indicating altered PUFA metabolism in maternal overweight. Additionally, maternal overweight was associated

predominantly with higher proportions of long-chain FAs (i.e. C18:1, C18:0, C16:0, C14:0) and lower proportions of very long-chain FAs (C22 or higher) incorporated into (glyco)sphingolipids, suggesting altered sphingolipid metabolism. Moreover, maternal BMI seemed to be associated with changes in BA metabolism and a disbalance between hydrophilic and hydrophobic BAs in CB.

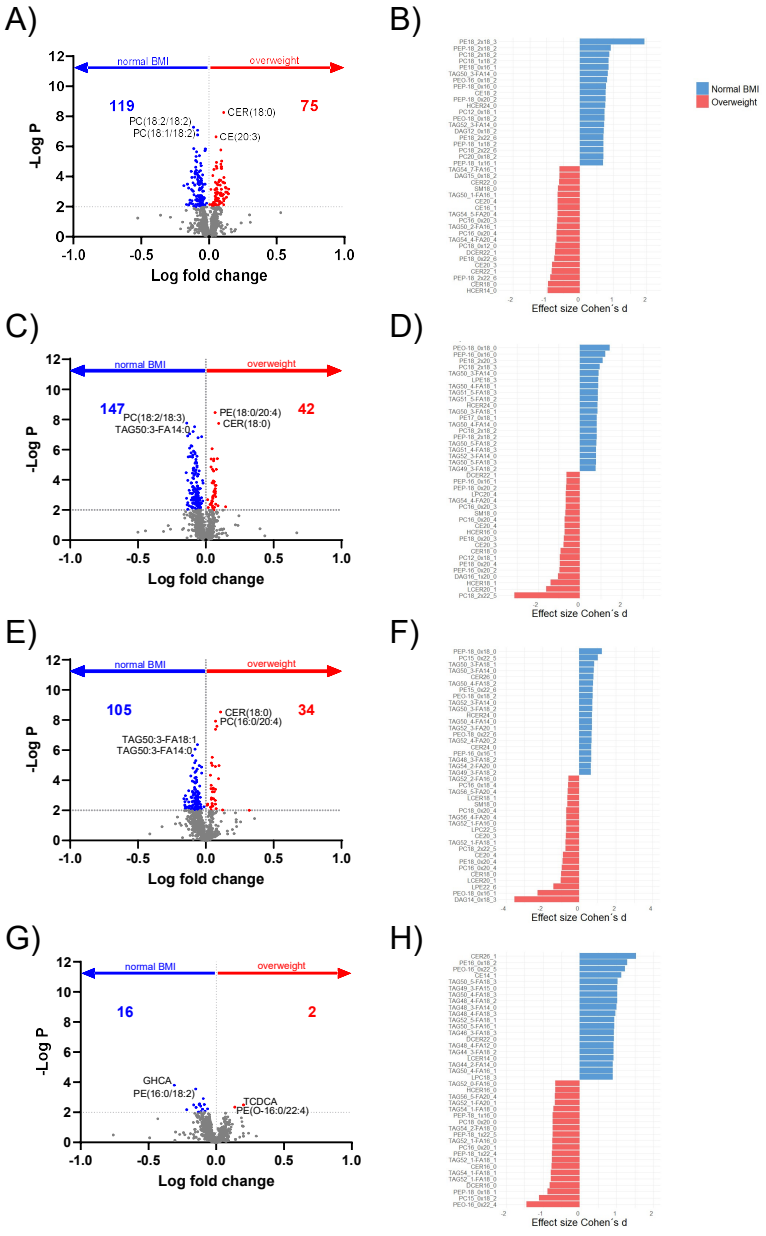

Figure 16: Differences in lipid species composition during pregnancy and in CB.
Differences in the composition of lipid species in the circulation of women with a normal BMI and overweight in A) 1st trimester, C) 2nd trimester, E) 3rd trimester and G) CB. Lipids that are significantly higher in lean individuals are highlighted in blue, whereas overweight-associated lipids are highlighted in red ($p < 0.01$ which equals -log$P > 2$). The respective 20 highest effect sizes (Cohen´s *d*) were calculated for B) 1st trimester, D) 2nd trimester, F) 3rd trimester and H) CB.

5. PUFAs and the sphingolipidome in maternal overweight

As a lipid signature characterized by altered PUFA lipids as well as low VLCFA sphingolipids for maternal overweight, PCA was performed with selectively these lipid species to achieve a clearer discrimination between women with overweight and lean individuals. Notably, we excluded CB for these analyses as the CB lipidome was distinct from the maternal.

5.2 Gestational PUFA metabolites and sphingolipidome discriminate between women with a normal BMI and overweight

Concentrations of maternal PUFA-containing lipid species that were linked to LA, AA, linolenic acid and C20:3 were used to perform PCA. As observed for PCA with all lipids (Part D, results, chapter 3.1), PUFA lipids could replicate a separation of the samples along principal component one (Figure 17A, B). Moreover, the separation was more pronounced with principal component two (Figure 17C). Intriguingly, LA species were positively associated with principal component two, whereas AA species displayed an opposite association (Figure 17D).

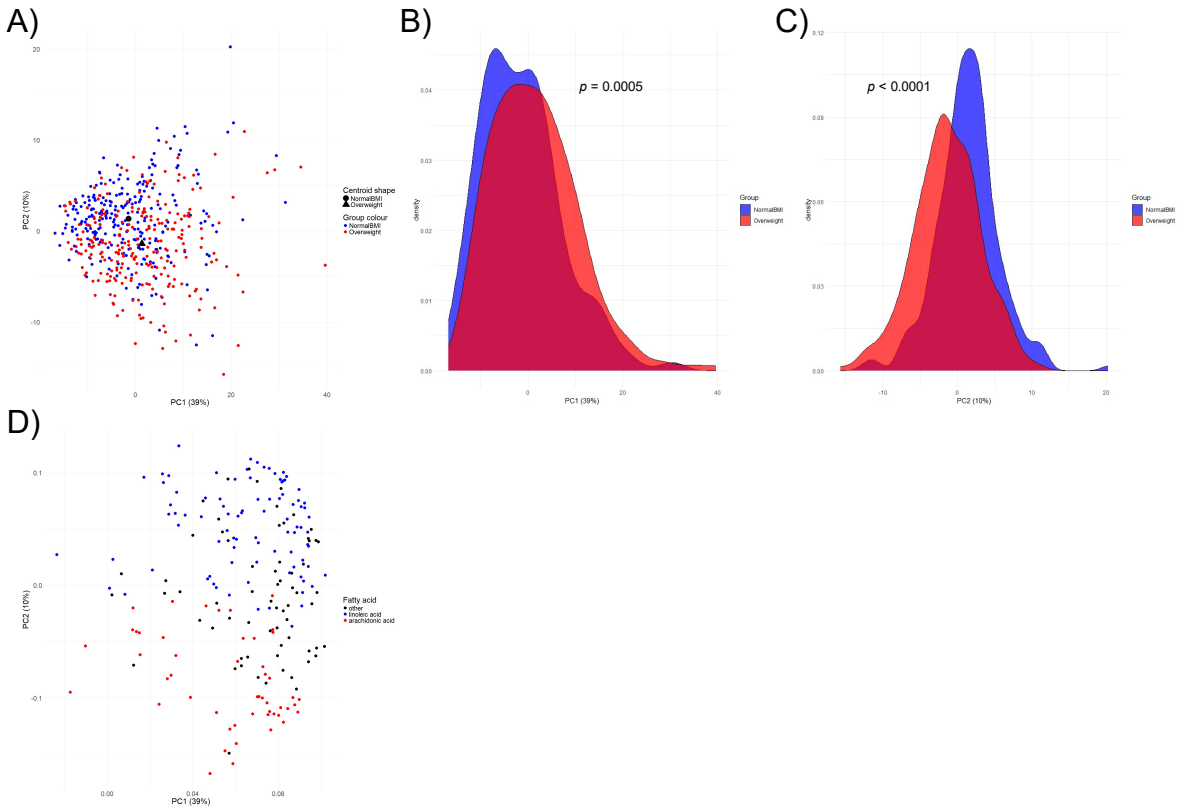

Figure 17: Principal component analysis with PUFAs
Principal component analysis (PCA) with concentration of all lipids containing either LA, linolenic acid, FA C20:3 or AA from the three trimesters without cord blood. A) Distribution of samples across principal component one and two. Samples from lean individuals are highlighted in blue, whereas those from women with overweight are red. Black circle and triangle represent centroids of normal BMI and overweight group, respectively. Estimated density across B) principal component one and C) two. D) Loading plot with linoleic acid and arachidonic acid containing lipids highlighted in blue and red, respectively.

In chapter 3.1 (Part D, results), maternal overweight was associated to an altered sphingolipid composition, characterized by lower very long-chain FAs compared to lean individuals. Thus, it was hypothesized that the composition of the sphingolipid pool could discriminate between lean and overweight individuals during gestation in PCA. The composition of the sphingolipidome was sufficient to discriminate between samples taken from the three trimesters as indicated by a distinct centroid location within the PCA plot (Figure 18A). Samples from women with overweight were positively associated with principal component one and two, whereas samples from lean individuals were negatively associated with these two principal components (Figure 18B, C). Notably, LCFAs (C14 to C20) were positively associated with both principal components, whereas VLCFAs were negatively associated with both principal components (Figure 18D). In summary, maternal overweight was associated to a distinct PUFA lipidome compared to lean individuals and a sphingolipidome characterized by lower VLCFAs.

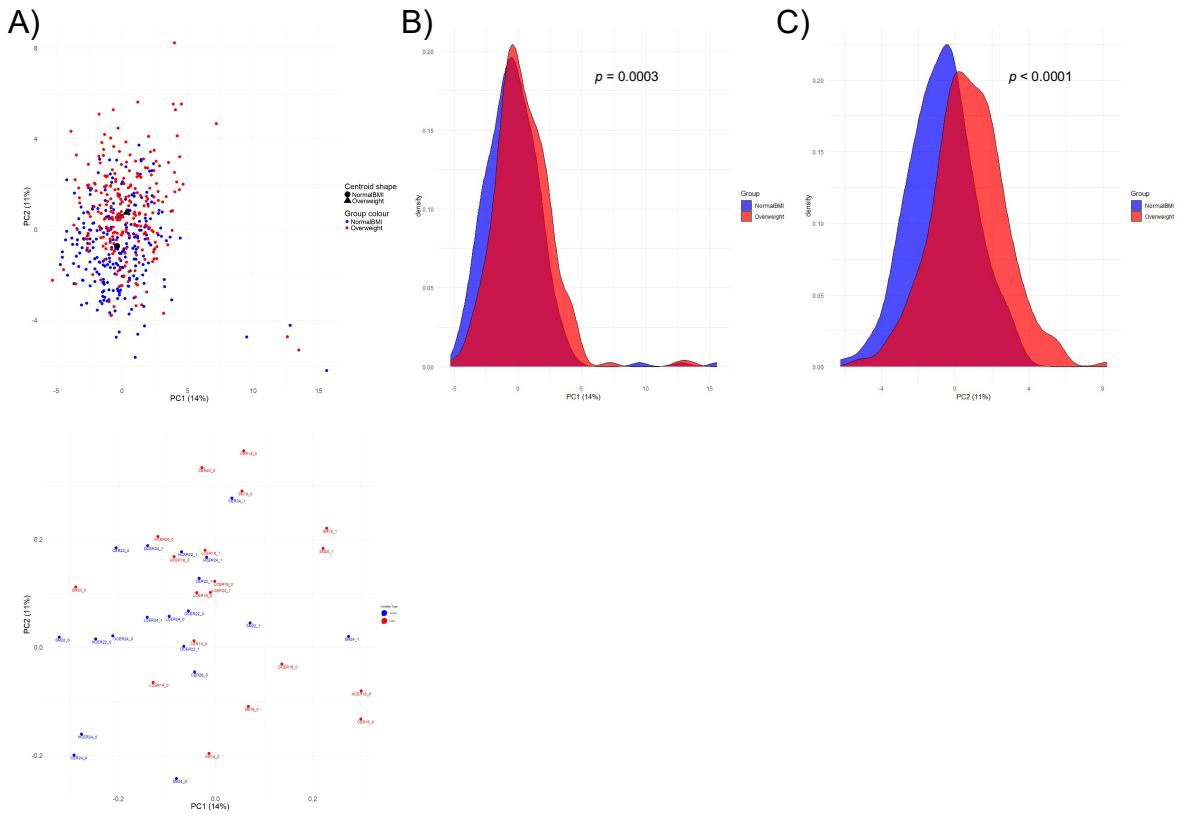

Figure 18: Principal component analysis with sphingolipids
Principal component analysis (PCA) with composition of sphingolipids from the three trimesters without cord blood. A) Distribution of samples across principal component one and two. Samples from lean individuals are highlighted in blue, whereas those from women with overweight are red. Black circle and triangle represent centroids of normal BMI and overweight group, respectively. Estimated density across B) principal component one and C) two. D) Loading plot with very long-chain FAs (VLCFA) and long chain FAs (LCFA) containing lipids highlighted in blue and red, respectively.

5.3 Maternal overweight is associated with higher FA desaturation and elongation

As we observed several PUFAs to be differentially abundant in samples from lean women and participants with overweight, we analyzed ratios of respective FA species to provide insights into endogenous FA processing (i.e. elongation and desaturation). Moreover, it was investigated if PUFAs could serve as a major discriminator between lean women and participants with overweight.

Firstly, it was analyzed if the total circulating LA and AA pool differs between lean women and participants with overweight to detect potential biases in consumption of dietary essential FAs. LA as a strictly essential FA from the diet was not different between the two groups during gestation. However, the CB lipidome of lean individuals contained more LA compared to participants with overweight (Figure 19A). In contrast, the maternal lipidome of women with overweight was characterized by higher AA content compared to lean individuals (Figure 19B). As we excluded higher dietary intake of LA in lean women, the ratio from LA to AA was analyzed. As expected, the circulating lipidome from lean individuals was characterized by a higher LA to AA ratio during gestation and in CB compared to women with overweight in all lipid classes (Figure 19C) and within phospholipids (Figure 19D). Moreover, this ratio increased in both groups during pregnancy (Figure 19C, D). However, although the LA to AA ratio in TAG showed similar associations with maternal BMI and gestational timepoint, LA to AA ratio in TAG was not significantly higher in CB from lean individuals (Figure 19E). Moreover, the ratio between LA and linolenic acid was higher in lean individuals in the first trimester compared to women with overweight but decreased during pregnancy, suggesting altered Δ6-desaturase activity (Figure 19F). The ratio between C20:3 and LA was used as a proxy for Δ5-desaturase activity and increased during pregnancy in both groups. Notably, the ratio was higher in lean women during late pregnancy (Figure 19G). The ratio of linolenic acid to C20:3 was used as a proxy for PUFA elongation. In the first and second trimester, this ratio was higher in lean individuals and increased during pregnancy in both groups. Notably, there was a statistical trend for a higher linolenic acid to C20:3 ratio in CB from lean individuals (Figure 19H). To sum up, maternal overweight was

characterized by a decreased LA to AA ratio in phospholipids and TAG. This was in line with higher ratios of FAs to their desaturated and elongated derivates in lean individuals compared to women with overweight, suggesting alterations of endogenous PUFA modifications (i.e. desaturation and elongation).

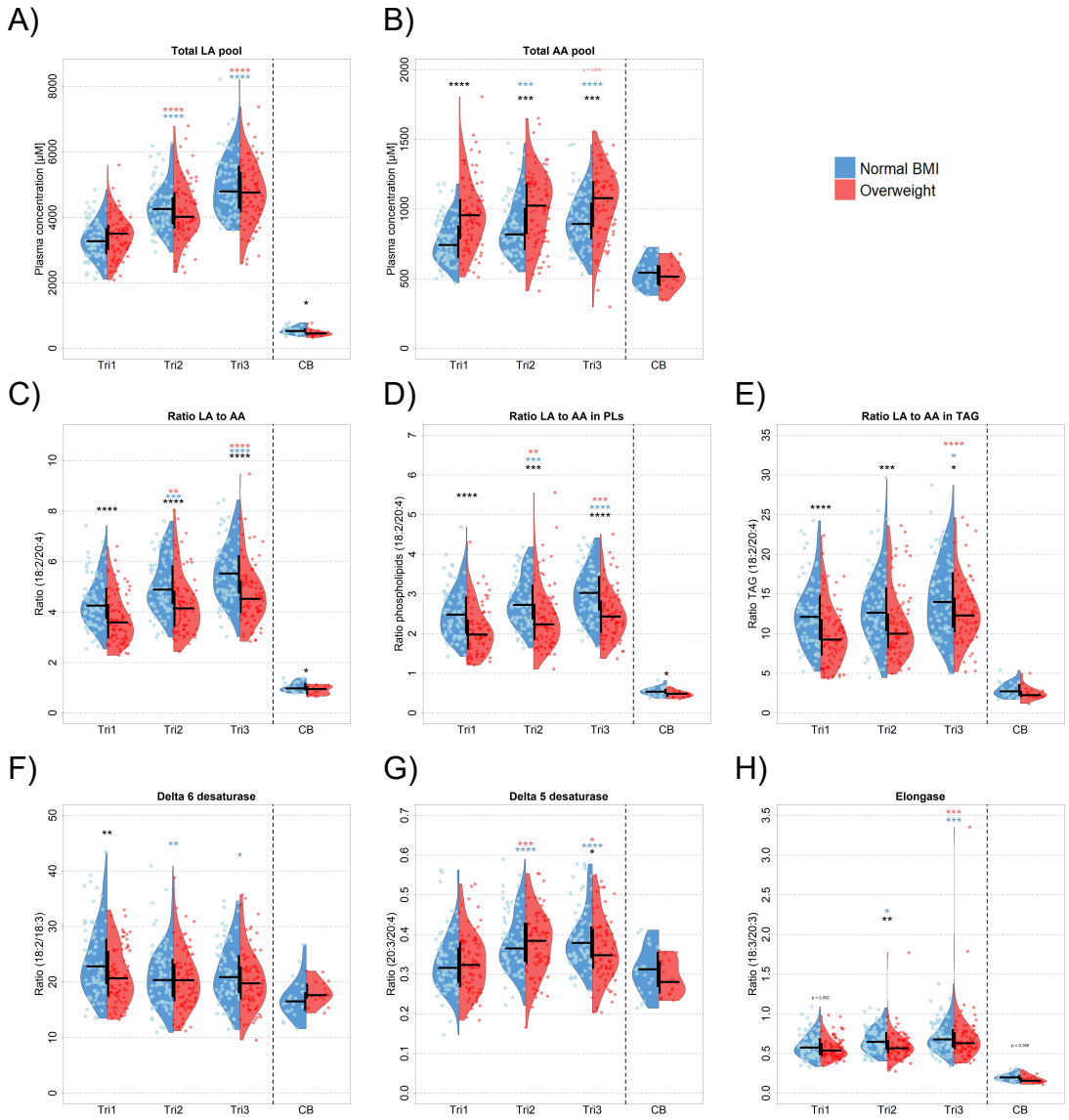

Figure 19: PUFA metabolism during pregnancy
Concentration of total A) LA and B) AA pool at each trimester and in cord blood. The ratio between LA and AA was calculated in C) all lipid classes, D) phospholipids and E) TAGs. Similarly, the ratios between F) LA and linolenic acid, G) C20:3 and AA as well as H) linolenic acid and C20:3 at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of groups. Black asterisks and *p* values represent difference between normal BMI and overweight as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

6. Maternal overweight is associated with altered CB BAs

Fetuses are capable of performing BA modifications such as hydroxylation and sulphation *in utero* (Colombo et al., 1987; Setchell et al., 1988; Wood et al., 2017). Moreover, these modifications lead to the synthesis of “unusual” BAs, which are usually not found in human adults and are considered “newborn-specific” (Seki et al., 2011; Wang et al., 2020). Further, CDCA can be modified to produce HCA (Bremmelgaard & Sjovall, 1980). As we observed high TCDCA and low levels of its hydroxylated derivate GHCA in CB from women with overweight compared to lean individuals, we aimed to characterize the maternal and CB BA pool more in detail with regard to CDCA-HCA metabolism.

6.1 CDCA and HCA species are altered in maternal overweight

In the maternal circulation, concentration of both conjugated CDCA species GCDCA (Figure 20A) and TCDCA (Figure 20B) as well as unconjugated CDCA (Figure 20C) were not associated with maternal BMI. Moreover, maternal levels of these CDCA species neither decreased nor increased during pregnancy (Figure 20A-C). However, taurine conjugated TCDCA was higher in the CB taken from women with overweight compared to lean individuals. Notably, TCDCA seemed to be more abundant in CB compared to maternal samples irrespective of maternal BMI (Figure 20B). When inspecting circulating concentrations of HCA species, exclusively GHCA (Figure 20D) was detected in almost all maternal samples, whereas THCA (Figure 20E) and HCA (Figure 20F) were mostly undetectable. Notably, GHCA significantly increased during the course of pregnancy in both groups (Figure 20D). Strikingly, all of these HCA species were detectable in almost all CB samples (Figure 20D-F). Notably, GHCA was higher in the CB taken from lean participants compared to women with overweight (Figure 20D). Additionally, the ratio of all CDCA to HCA species was calculated to provide insights into a potential hydroxylation mechanism. However, maternal BMI was not associated with altered ratios of all CDCA to HCA species at any timepoint that was analyzed (Figure 20G). In summary, maternal BMI was not associated with an altered ratio of CDCA to HCA species. However, the CB BA pool seemed to differ from the maternal BA pool characterized by a higher abundancy of HCA species.

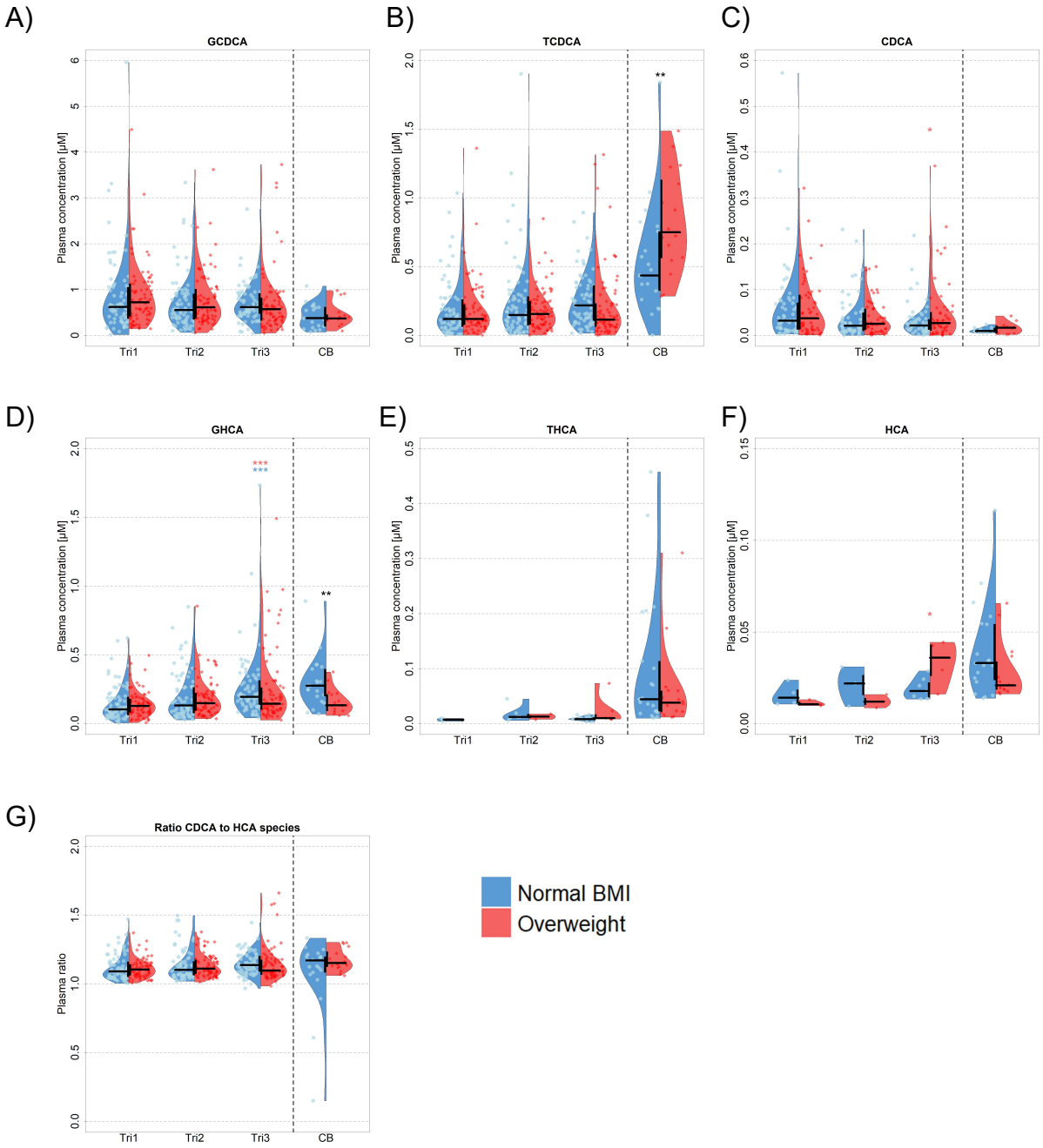

Figure 20: CDCA and HCA metabolites during pregnancy.
Concentration of A) GCDCA, B) TCDCA, C) CDCA, D) GHCA, E) THCA and F) HCA at each trimester and in cord blood as well as G) the calculated ratio between CDCA and HCA species. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of respective group. Horizontal black lines represent the median and vertical black lines represent the interquartile range for each group. Black asterisks and *p* values represent difference between normal BMI and overweight at the respective timepoint as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test with Bonferroni correction. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

6.2 CB-specific BAs and BA hydrophobicity are altered in maternal overweight

Since we observed differences between the gestational and CB BA pool, the association of maternal BMI with individual BA species was investigated. Thus, maternal BMI was correlated to the circulating concentration of BAs in third trimester (Figure 21A) and CB (Figure 21B). In the third trimester, maternal BMI correlated positively with THCA and HCA. Moreover, conjugated BAs strongly correlated with each other. However, the secondary BA 3-oxo-cholic acid (3-oxo-CA) was almost undetectable in the maternal circulation, thus there was not enough overlap with maternal BMI or other BAs to calculate correlation coefficients. Similarly, calculation of correlation coefficients between hydrophilic BAs were not determined (Figure 21A). Conversely, maternal BMI correlated negatively with 3-oxo-CA, GHCA and cholic acid-7-sulphate (CA-7S) and positively with TCDCA and TUDCA/THDCA. Moreover, hydrophilic BAs correlated with each other (Figure 21B).

Next, BA species that seemed rather CB-specific were assessed across the course of pregnancy and in CB. 3-oxo-CA and CA-7S were mostly undetectable in the maternal circulation. However, these BAs were higher in CB taken from lean individuals compared to women with overweight (Figure 21C, D, respectively). As observed for other hydrophilic BAs, muricholic acid species were mostly undetectable in maternal blood samples, but abundant in a subset of CB samples. However, CB muricholic acids were not associated with maternal BMI (Figure 21E, F). Due to the association of maternal BMI with CB but not maternal BA species, BA composition in the CB was analyzed. The cumulative proportion of hydrophilic HCA species (i.e. THCA, GHCA, HCA) in CB was approx. 8.5% in lean women (Figure 21G) and 6% in women with overweight (Figure 21H). Conversely, an increased cumulative proportion of hydrophobic CDCA species (i.e. TCDCA, GCDCA, CDCA) in CB was associated with maternal overweight. 19% of BAs in the CB from lean women belonged to this BA class, whereas almost one third of CB BAs were CDCA species in women with overweight (Figure 21G, H). In line with the altered BA composition, the BA pool was more hydrophilic in CB from lean individuals compared to CB from women with overweight. This association was exclusively detected in CB, but not

maternal samples. However, BA hydrophobicity decreased in both groups during gestation (Figure 21I).

Next, PCA was performed to analyze, if the concentration of BAs had potential to discriminate between CB samples from lean individuals and women with overweight. Notably, CB samples from lean women were positively associated with principal component two, while CB samples from women with overweight displayed an opposite association (Figure 21J). Due to statistical reasons (i.e. setting a minimum variance for all analytes to 0.1), only seven of the initially 37 BA species remained for PCA. Among these, rather hydrophilic BAs GHCA, THCA, GCA and TCA as well as the sulphated BA TLCA-3S positively contributed to principal component two. In contrast, hydrophobic BAs GCDCA and TCDCA were negatively associated with principal component two (Figure 21K). As HCA species are antagonistic and CDCA species are agonistic for FXR, it was hypothesized that FXR activity might be altered. Since phospholipid transfer protein (PLTP) and ABCB4 are transcriptional targets of FXR (L. Huang et al., 2003; Urizar et al., 2000), the correlation between phospholipids and BAs in CB was analyzed. Hence, hydrophilic BAs, in particular THCA and GHCA, were positively associated with CB PUFA-containing PLs, including LA esterified to PC and DHA esterified to PE. Consistently, hydrophobic CDCA species tended to display an opposite association with those lipids (Figure 21L).

To sum up, maternal overweight was selectively associated with an altered BA pool composition in CB characterized by less hydrophilic and CB-specific BAs compared to lean individuals. Moreover, these BAs were associated with CB PUFA-containing PLs, suggesting an altered transplacental transport of essential FAs.

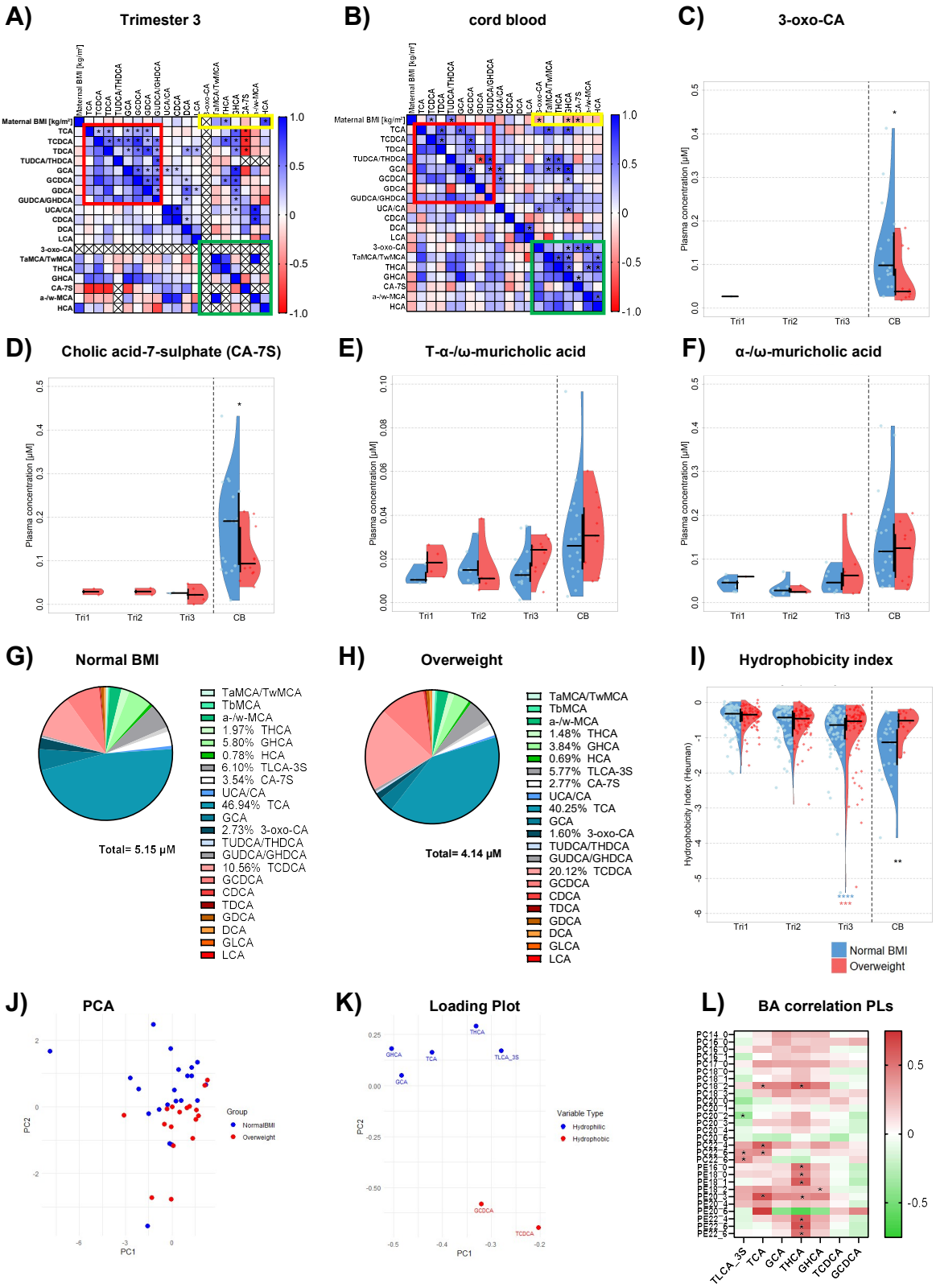

Figure 21: Bile acid metabolism is altered in cord blood.
Correlation of maternal BMI with bile acids (BAs) in A) 3rd trimester and in B) cord blood (CB). Longitudinal concentration of C) 3-oxo-CA, D) CA-7S, E) T-α-/ω-MCA and F) α-/ω-MCA. BA composition in CB from women with G) a normal BMI and H) overweight. I) Hydrophobicity index during pregnancy. J) Principal component analysis of CB samples with BAs and K) respective loading plot. L) Correlation of BAs with PLs in CB.

Part E: Discussion

1. GDF15 as placenta-derived immunometabolic link

We detected higher levels of GDF15 in lean women compared to individuals with overweight. Consistently, first trimester GDF15 levels were negatively associated with pre-pregnancy BMI in a previous study (Petry et al., 2018) and these findings are in line with the role of GDF15 in the suppression of appetite through its receptor GFRAL (L. Yang et al., 2017). Notably, a recent study has shown synergistic functions of GDF15 and leptin in appetite regulation in brain cells positive for both GFRAL and LepR (Breit et al., 2023). Similarly, GFRAL-LepR double positive cells might exist in the placenta to have synergistic signaling in a homologous pattern, although GFRAL is not abundantly expressed in placentas from macaques (Klein et al., 2023). However, studies focusing on non-pregnant individuals are showing either a positive (Sarkar et al., 2020; Tsai et al., 2015) or a negative association between BMI and serum GDF15 (Dostálová et al., 2009). Since gestational levels increased intensively during pregnancy and were not associated with late pregnancy BMI in our study (in line with Moore et al. (2000) and Wertaschnigg et al. (2020)), together with its high placental expression (Hromas et al., 1997; Marjono et al., 2003; Yokoyama-Kobayashi et al., 1997), the primary role of gestational GDF15 seems to be unrelated from regulation of appetite during pregnancy.

One option is the involvement of GDF15 in the regulation of lipid metabolism. As such, GDF15 upregulates gene expression of lipolytic genes (i.e. ATGL, HSL) in different AT depots (Chrysovergis et al., 2014). Consistently, muscles secrete GDF15 after exercise, which induces lipolysis in human AT (Laurens, Parmar, et al., 2020). Moreover, mouse experiments showed that circulating GDF15 increased after dietary FAs compared to vehicle or isocaloric glucose (Kanta et al., 2023; D. Wang et al., 2024). Consistently, we observed correlations between gestational GDF15 and FFA. Hence, placental GDF15 secretion might serve on the one hand as an autocrine mechanism to induce placental lipolysis and on the other hand as a paracrine mechanism to induce AT breakdown of lipid stores to release FFAs, which are subsequently shuttled to the fetus. In turn, FFAs might stimulate placental GDF15 secretion similarly to dietary FAs in previous studies. Notably, FFAs are implicated in

peripheral insulin resistance during pregnancy (Sivan et al., 1998), which also depends on length and number of double bonds of the respective FFAs (X. Chen et al., 2019). Hence, more detailed analyses focusing on the FA profile associated with increased GDF15 in lean pregnancies would further give insights into its role in peripheral insulin resistance. However, an autocrine function through GFRAL seems unlikely, since placental GFRAL mRNA is undetectable at least in macaques (Klein et al., 2023). Noteworthy, GDF15 increases profoundly during gestation (Dostálová et al., 2009). Thus, high levels of GDF15 might execute their signaling capacity at the placenta through other members of the TGF-β receptor superfamily, which are placentally expressed. However, mechanistical studies focusing on the direct effects of GDF15 with regard to placental lipid metabolism (e.g. in syncytiotrophoblasts) are lacking. Altogether, our data suggest that gestational GDF15 is rather associated with peripheral lipolysis than inducing lipolysis in a direct, autocrine fashion in placental cells.

Importantly, the function of gestational GDF15 might also be related to its initially proposed role as macrophage inhibitory cytokine, thus leading to an anti-inflammatory- phenotype (Bootcov et al., 1997). Consistently, high GDF15 levels interfere with checkpoint-based immunotherapies against cancer, whereas blockade of GDF15 improved immune cell infiltration into tumor microenvironment and therapy success in mice (Haake et al., 2023), thus demonstrating the immunosuppressive effect of GDF15. In parallel, this process might also be implicated in immunotolerance of the allogeneic fetus, since low serum levels of GDF15 were observed in women with recurrent pregnancy loss (Lyu et al., 2023). Altogether, these studies suggest an immunosuppressive role of GDF15. Notably, these pathological situations (i.e. cancer, gestational inflammation, stillbirth) are associated with overweight and obesity (Catalano & Ehrenberg, 2006; Challier et al., 2008; Field et al., 2001; R. Yao et al., 2014), underscoring a beneficial role of increased GDF15 levels in fetal antigen tolerance. However, data on the placental and uterine immunological microenvironment would give further insights into the immunomodulatory role of gestational GDF15.

2. TREM2 in gestational lipid handling

We observed an association of maternal overweight with TREM2 in late pregnancy. Consistently, TREM2 expression in AT of non-pregnant individuals correlated with BMI (Reich et al., 2023). Moreover, TREM2 was observed to be higher patients with NASH compared to control individuals (Hendrikx et al., 2022; Liebold et al., 2023). Collectively, these data point towards a role of TREM2 in lipid metabolism during pregnancy. However, the source of soluble TREM2 in our study is unclear. We detected a significant association between circulating TREM2 and maternal BMI exclusively in the third trimester. As the fetus and placenta massively grow in late pregnancy, soluble TREM2 in our study is probably related to high tissue remodeling and growth at that timepoint at the placenta. In line with that, the placenta consists of a higher percentage of TREM2-positive macrophages compared to other tissues (Deczkowska et al., 2020) and TREM2 is enriched in decidual compared to circulating macrophages (Gustafsson et al., 2008). As maternal obesity is characterized by larger placentas, increased macrophage infiltration and subsequent local inflammation compared to lean individuals (Bianchi et al., 2021; Challier et al., 2008), increased soluble TREM2 levels in this study might be indicative of placental size and inflammation. Hence, analysis combining TREM2 levels with ultrasound measures of placental volume or placental weight at birth could potentially underscore or falsify this hypothesis.

In contrast, the metabolic role of gestational TREM2 is barely described. Liu *et al*. (2023) concluded that soluble TREM2 in non-pregnant individuals is indicative both for cholesterol processing capacity of macrophages and local inflammation. Moreover, they observed a positive correlation of TREM2 with TAG and cholesterol and an inverse association with HDL (W. Liu et al., 2023). Consistently, we observed similar correlations between TREM2 and plasma parameters. Thus, the increase in gestational soluble TREM2 and its correlation with BMI in late pregnancy suggest alterations in cholesterol processing of placental macrophages in maternal overweight. Interestingly, CB TREM2 was positively associated with HDL, thus differing from the observation in the maternal circulation. The interpretation of this finding is challenging, since there are no studies investigating CB or fetal TREM2. Generally, TREM2 has the capacity to bind HDL and different apolipoproteins (W.

Song et al., 2017; Yeh et al., 2016). Noteworthy, fetal and maternal HDL differ substantially in their proteome including apolipoproteins (Sreckovic et al., 2013). This alters the affinity to TREM2 and, thus, might explain the differential association with circulating TREM2 that was observed in this thesis work.

Additionally, we hypothesized that gestational TREM2 is associated with circulating sphingolipids, since *in vitro* studies suggested that TREM2 binds SM (Y. Wang, Cella, et al., 2015). However, we did not observe an association between TREM2 and SM or other sphingolipids. Many studies that are dealing with the characterization of TREM2 focus on phagocytosis by microglia in neuropathological conditions (Takahashi et al., 2005). Noteworthy, sphingolipids are highly abundant in the central nervous system (Olsen & Færgeman, 2017). Hence, the association between TREM2 and sphingolipids becomes more important in neuropathological conditions than in our setting, where sphingolipid classes contribute less to the circulating lipid pool.

3. Endogenous PUFA metabolism is altered in maternal overweight

Mammalians are capable of converting LA to AA through a series of desaturation and elongation (Salem et al., 1999; Zaman et al., 2010). Nonetheless, it is of debate whether endogenous processing of LA contributes significantly to the systemic AA pool (Rett & Whelan, 2011). In the present study, it was observed that the LA pool from lean individuals and women with overweight within the maternal circulation was of similar size, whereas its putative derivate AA was significantly higher in women with overweight. Notably, it has long been known that LA is an essential FA as humans depend on exogenous LA intake for development. This was demonstrated by infant formulas lacking LA, causing several health issues that could be reversed by LA addition (Hansen et al., 1958). Consequently, this suggests that dietary intake of LA was similar between the two groups. In contrast, many previous studies reported increased circulating LA levels in maternal overweight or obesity (Al-Otaibi et al., 2020; Yu et al., 2022) or even a negative correlation with maternal BMI (Cinelli et al., 2016; Vidakovic et al., 2015). This discrepancy needs to be considered when translating findings between different studies.

Besides arising from endogenous desaturation and elongation of LA, its putative derivate AA could also be obtained from the diet (Taber et al., 1998). Thus, it cannot be excluded that the greater AA pool size in women with overweight derived from increased intake of AA. However, nutritional data from the present cohort argue against that hypothesis since PUFA intake of women with overweight was not higher compared to lean individuals (appendix, Table 3). Noteworthy, nutritional data were obtained from a 24-hour dietary recall in a prospective cohort and not during a long-term dietary study, thus reflecting recently consumed diet but not permanent nutrition of study participants. Hence, for more detailed conclusions on dietary patterns, long-term dietary recalls or analysis of the erythrocyte FA profile would provide a more exact overview. Despite of that, a 24-hour dietary recall is a useful and sufficient tool to estimate large differences in the selected cohort subgroups with regard to the aims of the current work.

Moreover, the ratios between intermediate FAs as proxies for desaturation and elongation of ω-6 FAs pointed towards increased elongase, Δ6- and Δ5-desaturase activity in women with overweight. Of note, for some of the FAs (e.g. linolenic acid, C18:3), the used method cannot discriminate between ω-6 and ω-3 FAs. Since there was no difference in the ratio of dietary ω-6 to ω-3 FAs between the two groups at any gestational timepoint (appendix, Table 3), a potential bias of higher ω-3 FA metabolism in one group seems unlikely.

Notably, studies analyzing maternal polymorphism of the enzymes executing the respective processes demonstrate their contribution to endogenous AA formation during pregnancy (de la Garza Puentes et al., 2017; Kawabata et al., 2023). Interestingly, individuals carrying a polymorphism of the gene encoding fatty acid desaturase 1 (FADS1) (the enzyme responsible for Δ5 desaturation of dihomo-γ-linolenic acid to produce AA), also displayed lower conversion from LA to γ-linolenic acid (Kawabata et al., 2023). However, the FA profile in the present cohort was rather associated Δ5-desaturase activity with increased elongation of linolenic acid than increased Δ6-desaturase activity in maternal overweight. Moreover, Kawabata et al. (2023) suggested that the AA to LA ratio is a better indicator for FADS1 gene polymorphism with regard to the whole ω-6 FA desaturation and elongation series than dihomo-γ-linolenic acid to AA. Notably, the latter displayed an association with

FADS1 polymorphism in the third but not first trimester (Kawabata et al., 2023), which is in line with the presented data on maternal overweight. In another study, increased gestational weight gain was associated with higher circulating dihomo-γ-linolenic acid, whereas LA showed no association, thus mimicking the adverse FA profile observed in maternal obesity (Vidakovic et al., 2015). As our cohort was matched for gestational weight gain, this process contributes less to our findings, although individual gestational weight gain might differ in our cohort. The increased synthesis of AA has the potential to alter the inflammatory milieu in the mother. For instance, after release from PLs by PLA2, AA induced apoptosis in a phagocytic cell line (Pérez et al., 2006). Hence, the observed alterations might be implicated in alterations of the maternal immune system, which was not addressed in the present study.

Moreover, the presented data suggest that gestational Δ5-desaturase activity is implicated in the synthesis of AA. This in line with the increased demand of AA for fetal brain development (Clandinin et al., 1980). The data of this thesis work suggest that Δ5-desaturase activity is increased in maternal overweight. With regard to this it would be interesting to see if the altered maternal FA profile in maternal overweight impacts offspring brain function and neuronal development. To be mentioned, maternal BMI was associated with alterations in fetal brain regions playing a role in the regulation of behavior (Norr et al., 2021), whereas maternal PUFA intake (i.e. ω-3) strongly influenced offspring behavioral outcomes (Middleton et al., 2018).

Collectively, the discussed data suggest alterations of endogenous PUFA metabolism during maternal overweight characterized by augmented desaturation and elongation of LA into AA. However, the implications of these findings on gestational and long-term health in mother and child require further investigation. Analysis of effect sizes further suggested that the described findings are presumably of biological relevance.

4. Phospholipids in CB and transplacental transfer of PUFAs

With regard to fetal supply with essential FAs, the importance of placental PL processing for the transport of PUFAs to the fetus was demonstrated previously (Gil-Sánchez et al., 2011; Powell et al., 2024). Notably, we detected higher PC and PE species enriched with AA in the circulation of women with overweight compared to control individuals, whereas those PL classes esterified to LA displayed an opposite pattern. Additionally, most lipids in CB were lower compared to maternal blood samples, whereas the hydrolyzed PC and PE derivates LPC, LPE and FFA were in a similar range or seemed to be even higher than in the maternal circulation. This further suggests the transplacental transport of particularly maternal PLs across the placenta for fetal PUFA supply. Notably, PUFA containing PLs that were characteristic for lean individuals were not identical between maternal circulation and CB. Whereas most of the PL species associated with a normal BMI possessed two PUFAs in the maternal circulation, CB PCs were additionally associated to tetradecanoic and palmitic acid. These two FAs are derived during DNL (Y. Lee et al., 2020), which suggests that hydrolyzed PLs are re-esterified after transplacental transport to FAs presumably derived during fetal DNL. Notably, dietary tetradecanoic acid (also known as myristic acid) and palmitic acid have synergistic effects with regard to lipotoxicity in murine hepatocytes (Martínez et al., 2015). A recent study has shown that tetradecanoic acid was higher in placentas from lean women compared to those from mothers with obesity (Ortiz et al., 2023), which is in line with the presented data on CB during maternal overweight. Another study found that maternal tetradecanoic acid incorporated into PLs was negatively associated with estimated fetal weight and abdominal circumference (L. J. Li et al., 2023). Apart from these studies, the role of tetradecanoic acid in the fetoplacental unit remains elusive.

In contrast, LA and linolenic acid incorporated into CB PLs were probably derived from the maternal circulation as they are essential FAs. Notably, *in vitro* studies suggest that villous cytotrophoblast cells, but not syncytiotrophoblasts from mothers with obesity have increased LA uptake compared to control subjects (Dubé et al., 2012). Although these data seem contradictory to the presented findings, it is unclear whether these cells secrete LA into the fetal circulation or further modify (i.e. desaturate or elongate) LA. Similarly, a study treating placentas *ex vivo* with

radiolabeled LA and DHA demonstrated an increased uptake and fetomaternal transport rate for placentas from women with overweight (Hirschmugl et al., 2021). Conversely, Gázquez et al. (2020) reported an increased transplacental transport of LA in lean women compared to individuals with obesity (Gázquez et al., 2020). However, all of the three studies used FFAs, which differs from the PL species found in this thesis work and the underlying transport mechanisms. Nonetheless, an increased placental uptake of lipids in maternal overweight seems likely and is in line with increased lipid deposition in placentas from women with overweight or obesity, which has been shown several times (Calabuig-Navarro et al., 2017; Cetin et al., 2012; L. Song et al., 2022). Notably, compared to healthy control women, CB from pregnancies complicated by gestational diabetes mellitus possessed less LA irrespective of BMI (Prieto-Sánchez et al., 2019), suggesting impaired LA transport across the placenta. Altogether, the presented data suggest that in healthy, physiological pregnancies (e.g. from lean women without gestational diabetes) PUFA-containing PLs are hydrolyzed at the placenta and re-esterified at the fetal side to saturated FAs that are presumably derived from DNL. In contrast to that, overweight-associated AA-containing PLs were higher in the maternal circulation, but not higher in CB samples from women with overweight. Of note, placentas from women with obesity have lower expression of Lysophosphatidylcholine Acyl Transferase 4 (LPCAT4), which is important for the re-esterification of FFAs to lysophospholipids (Powell, Uhlson, et al., 2023). Conversely, phospholipases PLA2G2A and PLA2G5 were increased in placentas from obese neonates compared to lean neonates (Varastehpour et al., 2006). As PLA2 activation is a critical step in the generation of lipid mediators, Varastehpour et al. (2006) suggested that placental PLA2 activation in maternal overweight might lead to an increased pro-inflammatory milieu. Analysis of lipid mediators (e.g. AA-derived prostaglandins) in CB and maternal circulation in the present cohort might give further insights into local inflammatory pathways and tracking of AA. Additionally, MFSD2A-mediated PUFA transport across the placenta was associated to CB alkaline phosphatase (ALP) (Prieto-Sánchez et al., 2019). Intriguingly, ALP serves as a canonical biomarker, among others, for disturbances of liver function and cholestatic conditions (Epstein et al., 1986). Thus, impaired PUFA transport across the placenta in PLs might be

related to fetal liver function and BA metabolism. The findings on CB BA metabolites during maternal overweight will be discussed in chapter 6 (Part E, discussion, below).

Importantly, this thesis work did not address whether the sex of the child impacts the capacity of transplacental lipid transport. Especially when it comes to maternal obesity, studies in mice showed that male fetuses from obese dams have decreased PL esterified to DHA and altered PL transporters compared to female fetuses (Powell et al., 2021). Similarly, the PL-PUFA profile in human placentas was differently affected by maternal obesity depending on fetal sex (Powell, Uhlson, et al., 2023). Although the subgroups in our cohort were matched for fetal sex, there was only a subset of CB samples (39 in total) available since not all participants gave birth at our institution. Hence, 12 from 22 (55%) of CB samples from lean individuals were female, whereas 8 from 17 fetuses (47%) were female in CB samples from individuals with overweight. Although the distribution was not statistically different (p = 0.898), it cannot be excluded that data on CB lipidomics underlie a fetal sex-dependent bias. Moreover, differences in maternal obesity were not evaluated because of the small CB sample size. Further analyses are needed to correct for fetal sex as a confounder in CB samples.

5. Sphingolipids maternal overweight

As already discussed in the introduction, blocking of ceramide synthesis reduces body weight and insulin resistance (Simon et al., 2019). Moreover, long chain ceramides (e.g. C14 to C18) are increased in AT from obese individuals, whereas high fat diet fed mice display an additional decrease of very long chain fatty acid (VLCFA) ceramides in AT (Turpin et al., 2014). Consistently, VLCFA ceramide species are inversely associated with liver cirrhosis score and predictive for survival rate in patients with cirrhosis (Grammatikos et al., 2015). In line with that, CER esterified to palmitate (C16:0) increased endoplasmic reticulum (ER) stress and DNL, while VLCFA ceramides showed an opposite effect under obesogenic conditions (Kim et al., 2019). Similarly, the synthesis of VLCFA ceramides promotes proliferation and cell survival *in vitro* (Karahatay et al., 2007; Mesicek et al., 2010). During pregnancy, reduced levels of VLCFA ceramides were observed in preeclampsia (Dobierzewska et al., 2017). A previous study has demonstrated that long chain and

very long chain ceramides are decreased in maternal overweight, whereas they observed an increase in SM esterified to palmitic acid compared to lean controls (León-Aguilar et al., 2019). Consistently, the present work could replicate most of these findings by León-Aguilar et al. (2019) and expand it by other VLCFA ceramides. As these VLCFA are preferentially synthesized by CerS (Levy & Futerman, 2010), the presented data suggest alterations of endogenous sphingolipid metabolism in pregnant women with overweight. However, the potential downstream effects of the altered sphingolipidome in maternal overweight with regard to insulin sensitivity, ER stress, and apoptosis in maternal and placental cells were not evaluated in the present thesis.

Noteworthy, León-Aguilar et al. (2019) observed that children of obese mothers “inherited” the above-described alterations in ceramide acyl chain length, which persisted until four years of age (León-Aguilar et al., 2019). It would be interesting to see, if this metabolic priming is also true for the additional sphingolipids that were detected in the current work. As such, altered levels of complex glycosphingolipids HCER and LCER were observed. Notably, a similar pattern compared to other sphingolipids was observed with regard to altered acyl chain length in maternal overweight. Notably, LCER was implicated in the production of ROS in mitochondria (García-Ruiz et al., 2000) and the activation of astrocytes (Chao et al., 2019), but acyl chain length was not further specified in both studies. Notably, LCER was reported to activate cytosolic phospholipase A2α (cPLA2α), thus contributing to AA release (Nakamura et al., 2013). The same group demonstrated similar results regarding SM and cPLA2α (Nakamura et al., 2010). This finding is of particular interest as the present work also observed altered levels of AA in maternal overweight. Combination of both results might provide further insights into maternal regulation of PUFA availability in the circulation by endogenous sphingolipid metabolism. With regard to AA release, synthesis of inflammatory lipid mediators comes into play. Intriguingly, LCER induces the AA-derivate prostaglandin E2 (PGE2) in amniotic epithelial cells (Moore et al., 2003), which was similarly shown for ceramide (Edwin et al., 1997), suggesting that LCER and ceramides in general contribute to immunometabolic crosstalk at the placenta. It would be interesting to see if the observed LCER species in the present work as well as other BMI-

associated sphingolipids have similar properties and if the acyl chain length conveys distinct signaling properties.

6. Bile acids

In this work, differences in maternal and CB BA pool were detected. As such, CB consisted of different BA species that were undetectable in most of the respective maternal samples and included mainly hydrophilic and sulfated BAs. Importantly, the fetus and placenta are able to execute these modifications already early in gestation (Colombo et al., 1987; Miki et al., 2002; Setchell et al., 1988; Wood et al., 2017). To be mentioned, these modifications are fundamental steps in the detoxification of cytotoxic, harmful substances including hydrophobic BAs (Alnouti, 2009; Kastrinou Lampou et al., 2023). This is in line with previous studies, demonstrating the existence of “unusual” and newborn-specific BAs (Seki et al., 2011; W. X. Wang et al., 2020). Noteworthy, only a few lipids were significantly different in CB samples from lean women and participants with overweight. Among these lipids, BAs seemed to be associated most with maternal BMI, displaying a shift from hydrophilic to hydrophobic BAs. Importantly, the concentration of seven BA species was sufficient to discriminate between CB samples from women with overweight and lean control women. Most of these BAs were CB-specific, indicators of BA detoxification and associated with maternal overweight, suggesting that fetal and/or placental BA detoxification is impaired in maternal overweight. Nonetheless, mechanisms mediating these observations remain elusive. One option might be that the placenta becomes less efficient to modify BAs during maternal overweight, but studies directly linking maternal overweight to placental BA processing are lacking. Of note, enzymes involved in the hydroxylation of BAs are members of the CYP450 enzyme family (Kastrinou Lampou et al., 2023). Similarly, another member of this family, Cyp1a1, is involved in the hydroxylation of 17β-estradiol at position 6α and shows decreased activity in placentas of obese women (Dubois et al., 2012; Spink et al., 1992). Notably, a major detoxification of CDCA into HCA requires the addition of hydroxylation at position 6α (Deo & Bandiera, 2008; T. Li & Chiang, 2014). In this work, it was observed that CDCA species were increased while HCA species were decreased in CB from mothers with overweight. However, it is unclear if Cyp1a1 uses CDCA as a substrate to synthesize HCA. Nonetheless, it is possible that other

members of the CYP450 enzyme family are regulated in a similar pattern as Cyp1a1 in maternal overweight and obesity. However, as described in the discussion (Part E, chapter 4), the proportions of fetal sexes were not similar between CB from control individuals and women with overweight. Hence, maternal obesity was shown to differentially alter placental steroid metabolism in male and female fetuses, which included enzymes of the CYP450 superfamily (Maliqueo et al., 2017).

Additionally, BAs possess a profound signaling capacity. While CDCA species are potent agonists of FXR, HCA species are strong antagonists (Lew et al., 2004; Zheng, Chen, Jiang, et al., 2021). Thus, we observed a shift from an antagonistic to an agonistic FXR milieu. FXR regulates the transcription of genes involved in several pathways, including phospholipid transport. As such, agonism of FXR increases the gene expression of phospholipid transfer protein (PLTP) and ATP binding cassette subfamily B member 4 (ABCB4) (L. Huang et al., 2003; Urizar et al., 2000). In line with that, endogenous FXR agonists (i.e. CDCA species) were inversely associated with phospholipids in CB, whereas antagonists (i.e. HCA species) displayed an opposite association in the current study. Since the concentration of these BAs is rather in a physiological than in a pathological range in CB, the signaling capacity on placental gene expression should be treated with caution. Similarly, their abundancy in placental cells in our cohort is unknown. Nonetheless, analysis of biological samples that are part of the enterohepatic circulation and thus exposed to higher levels of BAs (e.g. fetal liver, gallbladder or intestine) should be considered as further targets to detect potential BA signaling effects and the consequences on postnatal development. Notably, almost half of the meconial BA pool consisted of CDCA (29%) and HCA (19%) species while the proportion of HCA decreases rapidly after birth (Kimura et al., 1994). For instance, HCA species are regulators of systemic glucose homeostasis by a unique signaling capacity through the two main BA receptors FXR and TGR5 in enterocytes (Zheng, Chen, Jiang, et al., 2021). In addition, secondary BAs and in particular oxo-BAs have the potential to alter the differentiation of intestinal and colonic T cells, thus contributing to the regulation of intestinal immunity (Hang et al., 2019; X. Song et al., 2020). Collectively, the presented data suggest that maternal overweight interferes with fetal BA metabolism and detoxification with potential impact on transplacental transport of PUFAs.

All in all, the current thesis work expands the existing literature by a detailed overview on the circulating metabolome and lipidome during maternal overweight, and how maternal overweight presumably impacts placental and/or fetal lipid processing as well as detoxification. However, this work provides a correlative and descriptive overview on metabolic changes during maternal overweight but lacks mechanistical data to demonstrate the suggested mechanisms and implications.

Part F: Summary

Overweight is increasing worldwide and affects a respective percentage of women at reproductive age in modern societies. Pregnancy is a phase of tightly regulated metabolic and immunological adaptions to ensure fetal health. However, pregnancies complicated by maternal overweight are characterized by an altered lipid mobilization, low-grade inflammation, and subsequent gestational complications such as macrosomia, preeclampsia, and preterm birth. Importantly, the consequences go far beyond pregnancy itself since offspring from mothers with overweight are more likely to develop overweight and associated disorders themselves, thus being part of the transgenerational cycle of overweight. The circulating lipidome is dysregulated in overweight and was shown to play a pivotal role in the etiology of associated disorders. The present study aimed to define a metabolic signature during maternal overweight in a prospective pregnancy cohort. Thus, samples from each trimester and cord blood (CB) taken at birth were analyzed to determine the circulating metabolome.

Maternal overweight was characterized by lower gestational levels of GDF15, which correlated with maternal free fatty acids and sphingolipids. In turn, TREM2 was higher in women with overweight compared to lean individuals and was associated with triglycerides (TAG) and cholesterol levels.

Lipidomic analysis further revealed that polyunsaturated fatty acids (PUFA) incorporated into the highly abundant lipid classes phospholipids and TAG differed in the circulation of lean women and those with overweight. Maternal overweight was further characterized by an increased conversion of the essential fatty acid (FA) linoleic acid (LA) into arachidonic acid (AA) as indicated by ratios of intermediate FAs in this process. Additionally, very long chain FAs incorporated into different sphingolipid classes were higher in lean individuals compared to women with overweight. Notably, these alterations were part of the metabolic signature in maternal overweight throughout pregnancy as demonstrated by principal component analysis.

Strikingly, the CB lipidome was less affected by maternal BMI. Similar to the observations in the maternal circulation, CB from lean individuals was enriched with

phospholipids containing LA compared to participants with overweight, whereas AA was not altered. Additionally, bile acid (BA) metabolites were detected among the few lipids that were significantly altered between the two groups. Generally, hydrophilic BAs seemed to be specific for CB compared to maternal samples and were lower in CB from women with overweight. In contrast, hydrophobic BAs showed an opposite pattern, which altogether contributed to an increased hydrophobicity index of the BA pool specifically in CB but not maternal circulation.

In summary, the present work defines a lipidomic signature in maternal overweight, indicating an altered endogenous PUFA and sphingolipid metabolism. Additionally, alterations of phospholipids containing LA in CB point towards an altered transplacental transport in maternal overweight. Our data further suggests that BA detoxification is impaired in fetuses and/or placentas from women with overweight. Since many of the altered lipids have bioactive properties, future studies should address the potential impact of these findings beyond pregnancy for maternal and offspring health with regard to metabolic, immunological, and neurocognitive outcomes.

Part G: Zusammenfassung

Die Inzidenz an übergewichtigen Personen steigt weltweit, was zu einem vermehrten Auftreten von mütterlichem Übergewicht führt. Dabei ist es wichtig zu beachten, dass Schwangerschaften einer strikt geregelten Regulation von metabolischen und immunologischen Anpassungen unterliegen. Abweichungen von diesen physiologischen Anpassungen bei mütterlichem Übergewicht resultieren in einem veränderten Lipidstoffwechsel sowie einer peripheren, subklinischen Entzündung, infolgedessen es zu verschiedenen Schwangerschaftskomplikationen wie Makrosomie, Schwangerschaftsintoxikation oder Frühgeburten kommen kann. Epidemiologische Studien haben zudem gezeigt, dass Kinder von übergewichtigen und adipösen Müttern eine höhere Wahrscheinlichkeit haben Adipositas sowie die damit assoziierten Krankheitsbilder zu entwickeln, womit sie Teil eines transgenerationalen Kreislaufs in der Vererbung von Übergewicht werden. Darüber hinaus ist Übergewicht durch ein verändertes Lipidprofil im Blutkreislauf charakterisiert, welches stark mit der Entstehung von Übergewicht-assoziierten Krankheiten verknüpft ist. Daher ist das Ziel der vorliegenden Arbeit, einen metabolischen Fingerabdruck für mütterliches Übergewicht zu erstellen. Hierzu wurden Blutproben von jedem Trimester und dem dazugehörigen Nabelschnurblut hinsichtlich ihrer zirkulierender Metabolite untersucht.

Es konnte gezeigt werden, dass maternales GDF15 in normalgewichtigen Müttern höher war und mit freien Fettsäuren sowie Sphingolipiden korreliert. TREM2 hingegen zeigte eine erhöhte Abundanz in der Zirkulation von Übergewichtigen Müttern und konnte mit Triglyzeriden (TAG) und Cholesterin Metaboliten assoziiert werden.

Durch tiefgehende Analysen des zirkulären Lipidprofils konnte eine Veränderung von mehrfach ungesättigten Fettsäuren in den im Blut stark angereicherten Lipidklassen TAG und verschiedenen Phospholipiden detektiert werden. Diese Veränderungen konnten auf eine verstärkte Umwandlung der essenziellen Fettsäure Linolsäure in Arachidonsäure in übergewichtigen Müttern zurückgeführt werden, was durch das Verhältnis der Zwischenprodukte dieses Prozesses demonstriert werden konnte. Darüber hinaus besaßen normalgewichtige

Kontrollmütter vermehrt sehr langkettige Sphingolipide, wohingegen die Sphingolipid-Klassen von Müttern mit Übergewicht kürzere Fettsäureketten zeigten. Die dargelegten Abweichungen des Lipidprofils in übergewichtigen Müttern verglichen mit Kontrollmüttern wurden mittels Hauptkomponentenanalyse demonstriert und waren Teil des metabolischen Fingerabdrucks während der gesamten Schwangerschaft.

Überraschenderweise waren nur wenige Lipide im Nabelschnurblut zwischen den beiden Gruppen unterschiedlich. Analog zu den Veränderungen im mütterlichen Lipidom waren auch Phospholipide mit Linolsäure höher in Nabelschnurblutproben von normalgewichtigen Müttern, wohingegen Arachidonsäure unverändert war. Darüber hinaus waren insbesondere Gallensäuren im Nabelschnurblut mit mütterlichem Übergewicht assoziiert. Hierbei zeigten sich generell hydrophile Gallensäuren als Nabelschnur-spezifisch verglichen mit dem mütterlichen Gallensäurepool und zudem verglichen mit Nabelschnurblut von übergewichtigen Müttern. Hydrophobe Gallensäuren hingegen waren höher im Nabelschnurblut von Übergewichtigen, was insgesamt zu einem erhöhten Hydrophobizitätsindex führte. Zusammenfassend definiert diese Studie ein detailliertes, longitudinales Lipidprofil für mütterliches Übergewicht, welches einen veränderten endogenen Stoffwechsel von mehrfachungesättigten Fettsäuren und Sphingolipiden umfasst. Darüber hinaus scheint der transplazentale Transport der essenziellen Fettsäure Linolsäure in Phospholipiden in übergewichtigen Müttern verändert zu sein. Die dargelegten Daten deuten zudem auf eine beeinträchtigte fetale bzw. plazentale Detoxifizierung von Gallensäuren durch mütterliches Übergewicht hin. Viele der veränderten Lipide aus dieser Studie haben bioaktive Eigenschaften und können den Stoffwechsel vieler verschiedener Zelltypen verändern. Daher sind nachfolgende Studien nötig, um die Auswirkungen der dargelegten Ergebnisse über die Schwangerschaft hinaus für die Gesundheit von Mutter und Kind hinsichtlich metabolischer, immunologischer und neurologischer Konsequenzen zu überprüfen.

Part H: References

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Part I: Appendix

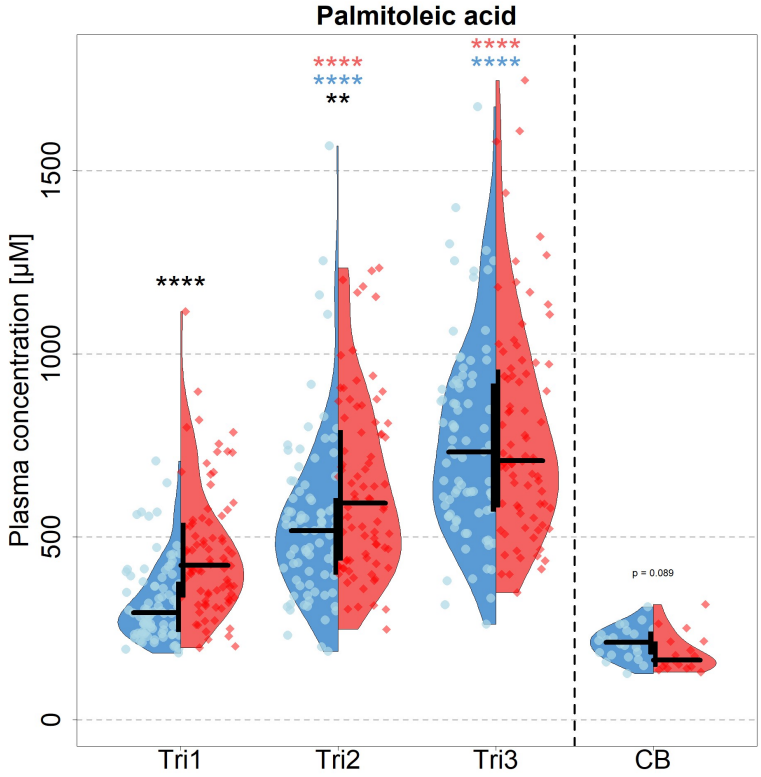

Figure 22: Palmitoleic acid during pregnancy
Concentration of palmitoleic acid at each trimester and in cord blood. Blue circles represent samples from lean individuals and red squares represent samples from women with overweight. Truncated violins represent the distribution of groups. Black asterisks and *p* values represent difference between normal BMI and overweight as determined by Student´s *t* test. Blue as asterisks and *p* values represent difference of the respective timepoint to first trimester in lean individuals, whereas red ones indicate difference in women with overweight as determined by paired *t* test. Tri1: 1st trimester; Tri2: 2nd trimester; Tri3: 3rd trimester; CB: cord blood; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Table 3: Dietary intake of selected FAs.

**p<0.01 as determined by Student´s t test between lean participants and women with overweight at each timepoint. Mean values ± standard error of the mean are shown. FA: fatty acid.

		Saturated FA (g) per 1000kcal	Monounsaturated FA (g) per 1000kcal	Polyunsaturated FA (g) per 1000kcal	Ratio ω6:ω3
Tri1	Normal BMI	17.08 ± 0.57	12.59 ± 0.46	7.12 ± 0.35	7.67 ± 0.54
	Overweight	18.19 ± 0.56	12.57 ± 0.36	5.79 ± 0.30 **	6.85 ± 0.48
Tri2	Normal BMI	17.93 ± 0.54	12.63 ± 0.40	6.01 ± 0.29	6.60 ± 0.50
	Overweight	18.46 ± 0.63	13.31 ± 0.49	5.33 ± 0.28	7.00 ± 0.45
Tri3	Normal BMI	17.74 ± 0.47	12.51 ± 0.43	5.88 ± 0.31	6.96 ± 0.48
	Overweight	18.73 ± 0.48	13.72 ± 0.49	6.19 ± 0.34	7.31 ± 0.48

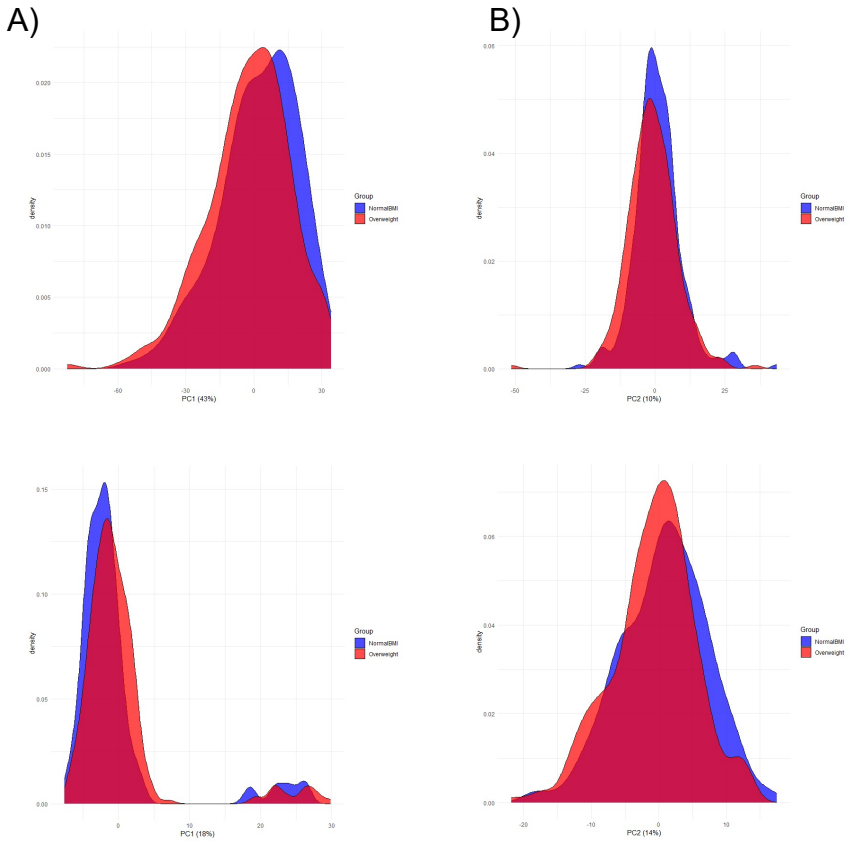

Figure 23: Principal component analysis of the lipidome from all timepoints
Estimated density during principal component analysis (PCA) with lipid species A), B) concentration and C), D) composition from the three trimesters and cord blood. Normal BMI group is blue, whereas overweight group is red. Estimated density across A, C) principal component one and B, D) two.

Table 4: List of hazardous substances according to GHS
Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Chemical	Manufacturer	Purity	GHS symbol	Hazard statement H	Precaution statement P
2-Propanol, LC-MS-Grade	Carl Roth	≥ 99,5 %	GHS02, GHS07	H225-H319-H336	P210-P261-P305 + P351 + P338
3-oxo-cholic acid (5β-cholanic aid-7α, 12α-diol-3-one)	Steraloids, Newport, UK		-	-	-
7α-hydroxycholesterol	Avanti Polaroids, Alabaster, USA	≥ 99%	-	-	-
7β-hydroxycholesterol	Avanti Polaroids, Alabaster, USA	≥ 99%	-	-	-
Acetonitrile	Merck, Darmstadt, Germany	≥ 99.9 %	GHS02, GHS07	H225, H302 + H312 + H332, H319	P210, P240, P302 + P352, P305 + P351 + P338, P403 + P233
Bovine serum albumin, BSA	Sigma-Aldrich, Taufkirchen, Germany	≥ 98%	-	-	-
Butylated hydroxytoluene, BHT	Sigma-Aldrich, Taufkirchen, Germany	≥ 99.0%	GHS09	H410	P273, P391, P501
Chenodeoxycholic Acid	Sigma-Aldrich, Taufkirchen, Germany	≥ 99%	-	-	-
Chloroform, Rotisolv, HPLC-Grade	Carl Roth	≥ 99,9 %	GHS06, GHS08	H302-H315-H319-H331-H336-H351-H361d-H372	P261-P281-P305 + P351 + P338-P311
Cholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-

Cholic acid-7-sulphate	Sigma-Aldrich, Taufkirchen, Germany		-	-	-
d4 Chenodeoxycholic acid	Cayman chemicals, Michigan, USA	≥ 95%	GHS07	H315, H319	-
d4 cholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
d4 Deoxycholic acid	Cayman chemicals, Michigan, USA	≥ 99%	GHS07	H302, H302, H302, H335	-
d4 Lithocholic acid	Cayman chemicals, Michigan, USA	≥ 99%	-	-	-
Deoxycholic Acid	Sigma-Aldrich, Taufkirchen, Germany	≥ 98%	-	-	-
Ethanol	Merck, Darmstadt, Germany	≥ 99.9%	GHS02, GHS07	H225, H319	P210, P233, P240, P241, P242, P305 + P351 + P338, P403 + P233
Formic acid	Sigma-Aldrich, Taufkirchen, Germany	≥ 98 %	GHS02, GHS05, GHS06	H226, H302, H314, H331	P210, P280, P301+P312, P303+P361+P353, P304+P340+P310
Glycochenodeoxycholic acid, GCDCA	Steraloids, Newport, UK	≥ 99 %	-	-	-
Glycocholic acid, GCA	Steraloids, Newport, UK	≥ 99 %	-	-	-
Glycodeoxycholic acid, GDCA	Steraloids, Newport, UK	≥ 99 %	-	-	-
Glycohyocholic acid	Steraloids, Newport, UK	≥ 99 %	-	-	-

Glycolithocholic acid, GLCA	Steraloids, Newport, UK	≥ 99 %	-	-	-
Glycoursodeoxycholic acid, GUDCA	Steraloids, Newport, UK	≥ 99 %	-	-	-
Hyocholic acid	Steraloids, Newport, UK	≥ 99 %	-	-	-
Hyodeoxycholic acid	Steraloids, Newport, UK	≥ 99 %	-	-	-
Lithocholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Methanol, Rotisolv, Ultra LC-MS-Grade	Merck, Darmstadt, Germany	≥ 99.97 %	GHS02, GHS06, GHS08	H225-H301+H311+H331-H370	P210 P270 P280 P303+P361+P353 P304+P340 P308+P311
NaCl	Sigma-Aldrich, Taufkirchen, Germany	≥ 99,5 %	-	-	-
NaOH	Sigma-Aldrich, Taufkirchen, Germany	≥ 97 %	GHS05	H290, H314	P234, P260, P280, P301 + P330 + P331, P303 + P361 + P353, P305 + P351 + P338
NH4Ac	Sigma-Aldrich, Taufkirchen, Germany	≥ 99%	-	-	-
Sulfuric acid	Sigma-Aldrich®, Munich	0,5 M	GHS05	H290-H314	P280-P303 + P361 + P353-P304 + P340 + P310-P305 + P351 + P338
Taurochenodeoxycholic acid, sodium salt	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Taurocholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Taurohyocholic acid	Steraloids, Newport, UK	≥ 99 %	-	-	-
Taurohyodeoxycholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Taurolithocholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Taurolithocholic Acid-3-Sulphate	IsoSciences, Pennsylvania		-	-	-
Tauroursodeoxycholic acid sodium salt	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Tauro-α-Muricholic acid	Steraloids	≥ 99%	-	-	-

sodium salt	Inc, Newport, UK				
Tauro-β-Muricholic acid sodium salt hydroscopic	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Tauro-ω-Muricholic acid sodium salt	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
Tween 20	Sigma-Aldrich, Taufkirchen, Germany	≥ 40%	-	-	-
Ursocholic acid	Steraloids, Newport, UK		-	-	-
Ursodeoxycholic Acid	Sigma-Aldrich, Taufkirchen, Germany	≥ 99%	-	-	-
Water, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS	Sigma-Aldrich, Taufkirchen	≥ 99.9 %	-	-	-
α-Muricholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
β-Muricholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-
ω-Muricholic acid	Steraloids Inc, Newport, UK	≥ 99%	-	-	-

Table 5: Kits

Kit/product	Manufacturer
Cholesterin FS determination kit	DiaSys Diagnostic Systems, Holzheim, Germany
HDL-C Immuno FS determination kit	DiaSys Diagnostic Systems, Holzheim, Germany
Human GDF15 ELISA Kit	R&D Systems, Minneapolis, USA
Human Leptin ELISA kit	R&D Systems, Minneapolis, USA
Human TREM2 ELISA kit	R&D Systems, Minneapolis, USA
Human/Canine/Porcine Insulin ELISA kit	R&D Systems, Minneapolis, USA
Internal Standard Kit for Lipidyzer™ Platform	SCIEX, Framingham, USA

Table 6: Buffers and solutions

Buffer/solution	Composition
Eluent A	• 20 mM NH4Ac • 1 mL formic acid in final 1 L H2O LC-MS grade
Eluent B	• 20 mM NH4Ac • 1 mL formic acid in final 1 L methanol/acetonitrile (1/3, v/v)
Methanol-BHT	• 500 mL methanol • 0.5 g BHT
Reagent Dilutent	1% BSA in PBS, pH 7.2-7.4
Washing buffer	0.05% Tween® 20 in PBS, pH 7.2-7.4

Table 7: List of consumables

Product	Manufacturer
96-well microplates	Sarstedt, Nümbrecht
Canules	Braun, Melsungen
Chromafil O-20/3, PTFE, Pore ø 0.2 µl, filter 3 mm	Machery-Nagel, Düren
Crimp cap, Teflon-coated	CS-Chomatographie Service GmbH, Langerwehe
Glas vials	Verex™ Vial, Crimp, µVial i3 (Qsert) Amber 51, Phenomenex, Aschaffenburg
HPLC column	Accucore Polar Premium 10x2.1 mm 2.6 µm Solid Core Defender Vorsäulen/Guards and Holder, Thermo Fisher Scientific, Waltham, USA
HPLC-column, Accucore™ Polar Premium	150 x 2.1 mm, 2.6 µm, Porengröße: 140 Å, Thermo Fisher Scientific, Waltham, USA
Metal beads	4 mm, Qiagen, Venlo, Netherlands
Phosphate buffered saline (PBS)	GIBCO/BRL, Eggenstein
Pipette tips	Sarstedt, Nümbrecht
Reaction tube, 1.5 ml, SafeSeal	Eppendorf, Hamburg
Reaction tube, 2 ml, SafeSeal	Eppendorf, Hamburg
Superose™ 6 10/300 GL column	GE Healthcare, Chalfont St Giles, UK
Syringes	Braun, Melsungen
UPLC-column	SecurityGuard ULTRA Holder for UPLC columns 2.1 to 4.6mm ID Phenomenex®, SecurityGuard ULTRA Cartridges UHPLC C18 für 2.1mm ID columns, Phenomenex, Aschaffenburg
UPLC-column, Kinetex XB-C18	150 mm x 2.1 mm x 1.7 µm, Phenomenex, Aschaffenburg

Part J: Register of figures

Part K: Register of tables

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Special thanks go to **Prof. Dr. Jörg Heeren** for giving me the opportunity to work on this project at the Institute of Biochemistry and Molecular Cell Biology of the University Medical Center Hamburg-Eppendorf. Especially, I really appreciate his trustful and supportive way of supervision during my time in the working group. Besides, I would like to thank Prof. **Dr. Petra Arck** for co-supervision of this work and **Prof. Dr. Nicola Gagliani** as part of the thesis committee.

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Curriculum vitae

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